Technical Bulletin 154 June 1942

The Pathogenicity and Genetics of Some Sorghum Smuts

Syed Vaheeduddin
Division of Plant Pathology and Botany

University of Minnesota
Agricultural Experiment Station

The Pathogenicity and Genetics of Some Sorghum Smuts

Syed Vaheeduddin
Division of Plant Pathology and Botany

University of Minnesota
Agricultural Experiment Station

Accepted for Publication, June 19, 1942

CONTENTS

The Pathogenicity and Genetics of Some Sorghum Smuts[1]

Syed Vaheeduddin[2, 3]

INTRODUCTION

SMUTS ARE among the most important diseases of sorghum. *Sphacelotheca sorghi* (Link) Clinton probably is the most destructive and is coextensive with the cultivation of the crop (6, 26, 28, 35). *S. cruenta* (Kühn) Potter is more or less common in East China (31), in some parts of India (6), and it occurs sporadically in the United States (35). *Sorosporium reilianum* (Kühn) McAlpine is more or less common in East China (31) and occurs sporadically in India (6), Egypt (5), Southern Europe (6), and the United States (35).

There long have been problems of controlling certain smuts by seed treatments, and breeding for smut resistance has therefore been attempted. In 1921 Zillig (51) showed that there were races of *Ustilago violacea* (Pers.) Fuck., differing in their parasitism for different members of the Caryophyllaceae, and physiologic specialization has been demonstrated subsequently for many cereal smuts also. Recently considerable work has been done to determine the nature and origin of physiologic races, and this was one of the objects of the present investigation.

Five physiologic races of *Sphacelotheca sorghi* are now known. The question arises, however, as to whether new ones may originate through mutation and hybridization. The present investigation is partly a continuation of that of Tyler (45), who observed differences in the pathogenicity of chlamydospores obtained by crossing various monosporidial lines obtained from a single chlamydospore.

[1] Presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Degree granted December, 1936.

[2] The writer wishes to acknowledge his gratitude and indebtedness to Dr. E. C. Stakman for suggesting this problem and for many valuable suggestions throughout the course of the investigation and in the writing of this manuscript. Thanks are also due to Dr. J. J. Christensen for many helpful suggestions during the course of the work and to Dr. Helen Hart and Dr. Ian W. Tervet for help in the preparation of the manuscript.

[3] Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 165-71-1-124, sponsored by the University of Minnesota, 1942.

In the present study extensive crosses were made between biotypes within *S. sorghi* to ascertain whether new physiologic races could arise in this way.

In many sorghum-growing regions of the world there are two or more smuts of sorghum, while in certain regions of Egypt and India as many as four have been found. Rodenhiser (36) demonstrated that *S. sorghi* and *S. cruenta* can hybridize, while Tyler and Shumway (47) proved this to be true for *S. sorghi* and *Sorosporium reilianum.* It was considered desirable to study the possible results of hybridization between *Sphacelotheca cruenta* and *Sorosporium reilianum* as well as between haploid biotypes of *Sphacelotheca sorghi.*

MATERIALS AND METHODS

THE ORIGINAL chlamydospore material of *Sphacelotheca sorghi* used in the experiments was obtained from Dr. L. J. Tyler,[4] and that of *Sorosporium reilianum* was collected in the summer of 1935 at the University of Minnesota Experimental Farm at Coon Creek, near Anoka, Minnesota.

Single chlamydospores and sporidia were isolated by the method described by Dickinson (10) and Hanna (17). The general method of designating smut materials follows: Capital letters A, B, C, D, etc., indicate chlamydospores; the Arabic numbers immediately preceding the letters indicate the cross or other source from which the spores were obtained; and the Arabic numbers immediately following the letters indicate the position of the sporidia, beginning with the tip cell, on promycelia produced by chlamydospores. Thus, 300A2 means the sporidium from the second cell of the promycelium produced by chlamydospore A resulting from Cross 300. $A2_1$ would indicate that two sporidia were budded from the second cell of the promycelium, the first being designated as A2 and the second as $A2_1$. When special symbols are used preceding the cross number, explanation will be made.

In studying the cultural characters of various monosporidial lines, potato-dextrose agar (1 per cent dextrose and 1.5 per cent agar) was used. Several nutrient media (Table 14) were used to induce sectoring. For comparative tests equal quantities of media were poured into 250 cc. Erlenmeyer flasks, autoclaved

[4] The writer wishes to acknowledge his thanks to Dr. Tyler, formerly of the University of Minnesota, for the supply of chlamydospore material obtained by inbreeding and hybridizing different biotypes of *Sphacelotheca sorghi*; and to Dr. H. A. Rodenhiser, U. S. Department of Agriculture, Washington, D.C., for the supply of chlamydospores of two physiologic races of *Sphacelotheca cruenta.*

simultaneously at 15 pounds pressure for 20 minutes, and cooled at room temperature. Notes were taken four to five weeks after the cultures were transferred to the flasks.

The sexual reactions of monosporidial lines of *Sphacelotheca sorghi* were determined by the Bauch test. This test was satisfactory for *S. cruenta* also but not for *Sorosporium reilianum*. The reliability of the method itself was tested by using three other criteria of sex differences: sporidial fusions, presence or absence of chlorosis in inoculated plants, and, finally, the production of chlamydospores.

Minnesota amber sorghum was used for inoculation studies as it is susceptible to the three species of smut studied. The seed was surface disinfected by soaking in a solution of 1 part of formaldehyde to 240 parts of water for 45 minutes. Then it was washed thoroughly in running water and dried. For greenhouse studies, 6 to 10 seeds were planted in 6-inch pots. When the seedlings were four to five weeks old they were inoculated by hypodermic needle with single and paired combinations of monosporidial lines that had been grown in potato-dextrose broth for 7 to 10 days, using about 75 cc. of broth in each flask. Before inoculation a suspension was made by adding an equal amount of sterile distilled water to the flasks containing broth culture. Seedlings were injected at two or three places in the stem to insure striking the growing points of the plants with the needle point.

For field studies the sorghum seeds were planted in hills in triplicate rows 40 feet long and 3 feet apart. Three to four seeds were planted in each hill, the distance between hills being one foot. The field studies were made at University Farm, St. Paul.

EXPERIMENTAL RESULTS

Sphacelotheca sorghi (Link) Clinton

Hybridization Between Biotypes

Source of material—Dr. L. J. Tyler isolated three chlamydospores from a single sorus of *S. sorghi* obtained from Amarillo, Texas, and designated them as Texas A, B, and C. When he mated different primary and secondary sporidia from chlamydospore Texas A, two sporidia from the third promycelial cell (A3 and $A3_1$) were compatible, apparently being of opposite sex. As a result of inoculating sorghum with A3 x $A3_1$ (Cross 22, Table 1) smutted heads with brown peridial walls appeared. When Tyler crossed one monosporidial line ($B2_1$) from chlamydospore Texas B

with another ($C2_3$) from chlamydospore Texas C, he obtained smutted heads with gray peridial walls. This cross was numbered 122. He then crossed monosporidial line 122B4 with Texas $A3_1$ and gave the resulting chlamydospore material to the writer, who designated this as Cross 123 and obtained four smutted heads with brownish-gray peridia. Inbreeding and outbreeding in these two crosses (i.e., 22 and 123) were then continued. Figure 1 illustrates the origin of crosses 22 and 123 and the crosses made afterwards.

The writer also obtained from Dr. Tyler chlamydospores of four crosses, representing brown x brown and brown x gray. These were numbered 2, 4, 6, and 7; table 1 illustrates the origin of these four crosses. Crosses 2 and 4 were made among four monosporidial lines from the promycelium of one chlamydospore 22A of the brown type; while crosses 6 and 7 were made between monosporidial lines from chlamydospore 22A (of brown type) and monosporidial lines from chlamydospore 122B (of gray type). All the smutted heads from Cross 4 were brown, while some were brown and some gray from crosses 2, 6, and 7. The writer studied monosporidial lines isolated from four chlamydospores from each head type of each cross, except Cross 4, which had only one type. For convenience, the Roman numeral I is used to designate gray heads, and II, brown heads. Thus I6 refers to gray heads of Cross 6 and II6 refers to brown heads of Cross 6, etc. Otherwise the designations are those given in the section on materials and methods.

Segregation of factors for cultural characters in *Sphacelotheca sorghi*—Dickinson (12) found that factors for cultural characters in *Ustilago hordei* and *U. levis* segregated on a 2:2 or 4:0 basis and that the segregation might take place either in the first or second nuclear division in the promycelium. He also found that the segregation of factors for cultural characters could be

Table 1. Crosses Made Between Monosporidial Lines of *Sphacelotheca sorghi* for Studying the Inheritance of Peridial Wall Color

Cross No.	f parents*	Peridial color in smutted heads resulting from cross
22	Texas A3 x Texas $A3_1$	Brown
122	Texas $B2_1$ x Texas $C2_3$	Gray
123	Texas $A3_1$ x 122B4	Brownish gray
2	22A1 x 22A4	33 brown; 1 gray
4	22A2 x 22A4	25 brown
6	22A3 x 122B4	9 brown; 28 gray
7	22A4 x 122B2	8 brown; 32 gray

* Chlamydospores Texas A, B, and C were obtained from a single sorus of a Texas collection.

independent of the segregation of factors for sex. Hanna and Popp (19) and Holton (20) obtained similar results with *U. avenae* and *U. levis*. Christensen (9) found that the segregation of factors for cultural and sexual characters in *U. zeae* could be on a 4:0, 3:1, 2:2, 1:2:1, or 1:1:1:1 basis. Rodenhiser (36) obtained segregation ratios of 2:2 and 1:3 for sex and 1:1:1:1 for color in *Sphacelotheca sorghi*. In *S. cruenta* he obtained ratios of 2:2 and 1:3 for sex and 4:0 and 1:1:1:1 for color. Tyler (45) obtained ratios of 2:2, 2:1:1, 3:1, and 4:0 for cultural characters in *S. sorghi* and stated that factors for cultural characters segregated independently of those for sex.

The writer studied cultural characters of monosporidial lines from 28 chlamydospores of crosses 2, 4, 6, and 7; and segregation ratios of 4:0, 3:1, 2:2, 1:2:1, and 1:1:1:1 were found (Tables 5 and 6).

Segregation of factors for sex—Kniep (24) in 1919, working with *Ustilago violacea,* showed for the first time that fusion occurs only between certain sporidia of the same promycelium and considered those that fused to be of opposite sex. Evidence of heterothallism in other smut fungi was later reported by Bauch (2), Stakman and Christensen (41), Dickinson (11), Hanna and Popp (19), Holton (20), Flor (14), and Rodenhiser (36).

Bauch (2) in 1923 found that the sporidia in *U. longissima* belong to three sex groups and later reported many sex groups in this species (3). Dickinson (11), working with *U. levis* and *U. hordei,* found that the sporidia on the promycelium of a single chlamydospore fell into two sex groups. Hanna and Popp (19) obtained similar results with *U. avenae* and *U. levis*. Hanna (18), working with *U. zeae* and *Sorosporium reilianum,* found that the four sporidia on certain promycelia of *U. zeae* and *S. reilianum* fell into two sexual groups and those on others belonged to four sex groups. Christensen (8) found 24 sexual groups in *U. zeae* when he inoculated corn seedlings with 37 monosporidial lines in different combinations. He concluded that there apparently were multiple factors for sex in *U. zeae*. Flor (14) obtained indications of a number of sexual groups in *Tilletia tritici* and *T. levis*. Rodenhiser (36) reported two sex groups in *Sphacelotheca sorghi* and *S. cruenta,* and he (37) later reported as many as nine sexual groups in *S. sorghi* when he combined in all possible combinations the monosporidial lines obtained by isolating the primary sporidia from the basal promycelial cell of each of 10 chlamydospores. Isenbeck (21), too, found that the sporidia on a single promycelium of *S. sorghi* fell into two sex groups. Tyler (46) found three

Table 2. Segregation of Factors for Sex on Each of Seven Promycelia of Seven Chlamydospores Resulting from Two Intraspecific Crosses of *Sphacelotheca sorghi*

II2A

	1	2	3	4	Sexual group
1	−	+	+	+	1
2	+	−	+	+	2
3	+	+	−	−	3
4	+	+	−	−	3

II6A

	1	2	3	4	Sexual group
1	−	+	−	+	1
2	+	−	+	+	2
3	−	+	−	+	1
4	+	+	+	−	3

II6B

	1	2	3	4	Sexual group
1	−	+	−	+	1
2	+	−	+	−	2
3	−	+	−	+	1
4	+	−	+	−	2

II6C

	1	2	3	4	Sexual group
1	−	+	+	+	1
2	+	−	+	+	2
3	+	+	−	+	3
4	+	+	+	−	4

I2A

	1	2	3	4	Sexual group
1	−	+	+	−	1
2	+	−	+	+	2
3	+	+	−	+	3
4	−	+	+	−	1

I2C

	1	2	3	4	Sexual group
1	−	+	+	−	1
2	+	−	−	+	2
3	+	−	−	+	2
4	−	+	+	−	1

I6B

	1	2	3	4	Sexual group
1	−	+	+	−	1
2	+	−	−	+	2
3	+	−	−	+	2
4	−	+	+	−	1

sex groups in *S. sorghi* when he paired two primary and three secondary sporidia from a single promycelium, "secondary sporidia" in this case referring to those that were produced on a promycelial cell after the first formed ones were removed.

Further investigation of sex in *S. sorghi* was undertaken. The sex of the monosporidial lines mentioned in the previous section was determined. Monosporidial lines from the same promycelium

Table 3. The Results of All Possible Pairings Between 24 Monosporidial Lines from Promycelia of Six Chlamydospores of *Sphacelotheca sorghi* Obtained from Sori with Brown and with Gray Peridial Walls and Produced from Two Separate Crosses

		II2A				II6A				II6B				II6C				I2A				I2C				Sexual Group
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
II2A	1	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	1
	2	+	−	+	+	+	+	+	−	+	+	+	−	+	+	+	−	+	−	+	+	+	+	−	+	2
	3	+	+	−	−	−	−	−	+	+	+	−	+	−	−	−	+	−	+	−	−	−	+	+	−	3
	4	+	+	−	−	−	−	−	+	−	+	−	+	+	+	−	+	−	+	−	−	−	+	+	−	4
II6A	1	+	+	−	−	−	+	−	+	+	+	−	+	−	+	−	−	−	+	+	−	−	+	+	−	5
	2	+	+	−	−	+	−	+	+	−	−	+	−	−	−	−	−	−	−	+	−	+	−	+	−	6
	3	+	+	−	−	−	+	−	+	+	+	−	+	−	+	−	+	−	+	−	−	−	+	+	−	7
	4	+	−	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	8
II6B	1	+	+	+	−	+	−	+	+	−	+	−	+	−	+	−	+	−	+	+	−	−	+	−	−	9
	2	+	+	+	+	+	−	+	+	−	+	−	−	−	+	−	−	+	−	+	+	+	−	−	+	10
	3	+	+	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	−	−	−	−	+	+	−	11
	4	+	−	+	+	+	−	+	−	+	−	+	−	−	+	−	−	−	−	+	−	+	−	−	+	12
II6C	1	+	+	−	+	−	−	−	+	−	−	−	−	−	+	+	+	−	+	−	−	+	+	+	−	13
	2	+	+	−	+	+	−	+	+	+	+	+	+	+	−	+	+	−	+	+	−	−	+	+	−	14
	3	+	+	−	−	−	−	−	+	−	−	−	−	+	+	−	+	−	+	−	−	−	+	+	−	15
	4	+	−	+	+	−	−	+	+	+	−	+	−	+	+	+	−	−	−	+	+	+	−	−	+	16
I2A	1	+	+	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	+	+	−	−	+	−	−	17
	2	−	−	+	+	+	−	+	+	+	−	−	−	+	+	+	−	+	−	+	+	+	+	−	−	18
	3	+	+	−	−	+	+	−	+	+	+	−	+	−	+	−	+	+	+	−	+	−	+	+	+	19
	4	+	+	−	−	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	−	+	+	−	20
I2C	1	+	+	−	−	−	+	−	+	−	+	−	+	+	−	−	+	−	+	+	−	−	+	+	−	21
	2	−	+	+	+	+	−	+	−	+	−	+	−	+	+	+	−	+	+	−	+	+	−	−	+	22
	3	+	−	+	+	+	+	+	−	−	−	+	−	+	+	+	−	−	−	+	+	+	+	−	+	23
	4	+	+	−	−	−	−	−	+	−	+	−	+	−	−	−	+	−	−	+	−	−	+	+	−	24

were paired in all possible combinations and tested on Bauch's medium, which was successfully used by Bauch (3, 4) in determining sex differences between monosporidial lines of *Ustilago violacea, U. zeae,* and *U. scorzonerae.* According to Bauch, when lines of opposite sex are mixed together on slightly alkaline malt extract agar (3 per cent malt extract, 2 per cent agar) they produce "Suchfäden" and "Wirrfäden" as a result of sporidial fusion and thus form a white mycelial mat on the culture (Plate 1). The writer obtained good results, even with transfers from old cultures, when the medium used by Bauch was slightly modified. The modified proportions of ingredients used were 35 g. of malt extract and 40 g. of agar to 1000 cc. of distilled water made alkaline by adding 16 cc. of normal sodium hydroxide per liter. Poured plates of this medium were inoculated in duplicate series with four monosporidial lines in the center and different possible combinations towards the periphery of the plate. The plates were incubated at 27½° C. for 48 hours. Combinations of the lines of opposite sex produced a white mycelial mat on the

surface of the colony (Plate 1). The writer observed two types of mycelial mat. In one type the mycelial growth was dense and covered the entire culture. For example, when all four sporidia from chlamydospore 23A were united in four combinations among one another, the combination 1x3 (Plate 1) produced dense mycelial growth and combination 1x4 produced a sparser growth.

Sorghum seedlings were inoculated "hypodermically" with the same combinations of monosporidial lines as those used for the Bauch test. Within six or eight days after inoculation, plants inoculated with combinations that were compatible in the Bauch test developed chlorosis, and, on maturity, smut appeared. This confirms the work of Tyler (45, 46) showing that the Bauch test is reliable for distinguishing sexual compatibility in *Sphacelotheca sorghi.*

On the basis of mating sporidia from only one promycelium, the sporidia on some promycelia are of two sex groups, while on others they are of three or four groups. Segregation for sex factors in promycelia from seven chlamydospores is shown in table 2. Sporidia on promycelia of chlamydospores II6B, I2C, and I6B fall into two sex groups; those of chlamydospores II2A, II6A, and I2A fall into three groups; and those of chlamydospores II6C fall into four groups. The segregation ratios are 2:2, 1:2:1, and 1:1:1:1 (Table 6).

When 24 monosporidial lines isolated from six chlamydospores were paired in all possible combinations on Bauch's medium, there was evidence of 24 sex groups (Table 3). Furthermore, when 74 monosporidial lines isolated from 28 chlamydospores of four crosses of the brown and gray groups were tried by the Bauch test, there was evidence of at least 60 sex groups.

A random sample of paired lines that reacted positively in the Bauch test were injected into sorghum seedlings and all produced chlorosis and eventually chlamydospores. This again establishes the reliability of the Bauch test for *S. sorghi* and confirms the work of Rodenhiser (36) who stated that chlorosis could be taken as an index of sex compatibility between monosporidial lines of *S. sorghi* and *S. cruenta.* Some of the paired lines which showed a negative reaction in the Bauch test were injected into sorghum seedlings, and 38 monosporidial lines were also injected singly into plants. None of the paired lines with negative reaction in the Bauch test produced chlorosis or chlamydospores (Plate 2). Out of the 38 monosporidial lines injected singly, however, two lines (II4B1 and II7C1) produced chlorosis on leaves and later sori in the inflorescences, while the others did not. Thinking that

these two lines might be solopathogenic, like those obtained by Christensen (9) and Eddins (13) in *Ustilago zeae,* four chlamydospores from sori produced by each line were germinated and sporidia were isolated and tested. None produced chlorosis alone, but compatible combinations produced typical chlorosis. Ten sporidia were then isolated from a test tube culture of line II 7C1 and the cultures inoculated singly into sorghum seedlings. Three out of ten caused infection alone while the others did not, although, when paired with certain others, chlorosis resulted. As most of the sporidia isolated from test tube cultures were haploid, those which caused infection alone might be either diploid or dicaryotic, and reduction or dissociation might have taken place in some sporidia in the process of budding in the culture. There is, of course, the possibility of mixture in the test tube or in the syringe at the time of the inoculation. As these lines had behaved like haploid lines in the Bauch test, however, it seems unlikely that there was either a mixture of lines or dicaryotic sporidia at that time. Further, it would be hard to explain why these lines, if diploid, would have differed in their behavior with various haploid lines. Unfortunately, therefore, the reasons for the unusual behavior of these lines cannot be given definitely. However, there were at least 60 sex groups among the 74 monosporidial lines. Thomas Laskaris later corroborated the writer's results with a large random sample of these lines.

Pathogenicity of combinations of monosporidial lines—Shumway (38) observed different degrees of chlorosis on corn and sorghum leaves when he inoculated with different compatible combinations of monosporidial lines of *Sorosporium reilianum.* In case of severe chlorosis he noticed that the leaves were killed, but in case of moderate chlorosis the mottling gradually disappeared and no gall was formed on such leaves. The writer (48) also observed different degrees of chlorosis when different f_1 lines of *Sphacelotheca sorghi* x *Sorosporium reilianum* were inoculated hypodermically into sorghum seedlings.

When the writer made several hypodermic inoculations into sorghum seedlings with different paired combinations of monosporidial lines of *Sphacelotheca sorghi* isolated from 28 chlamydospores of four crosses, he observed different degrees of chlorosis resulting from inoculation with different combinations (Table 4). In some cases the chlorotic areas were large and caused distortion of the leaves; in others the spots were numerous and scattered, did not cause distortion, and sometimes disappeared later; when chlorosis was "weak," the spots were small and disappeared after

Table 4. The Chlorotic Effect Produced by Monosporidial Lines of *Sphacelotheca sorghi*, Singly and in Various Combinations, as an Indicator of Sexual Compatibilities (Compare with Results in Table 3)

Number	Sporidial combinations	Chlorosis*
	WITHIN GROUP II	
1	2A1 x 2A2	S
2	2A1 x 2A3	M
3	2A1 x 2A4	M
4	2A2 x 2A3	W
5	2A2 x 2A4	W
6	2A3 x 2A4	W
7	2B2 x 2B3	S
8	2B2 x 2B4	S
9	2B3 x 2B4	O
10	2C1 x 2C2	S
11	2C1 x 2C3	O
12	2C2 x 2C3	M
13	2D2 x 2D3	W
14	2D2 x 2D4	O
15	2D3 x 2D4	M
16	4A1 x 4A2	S
17	4A1 x 4A3	M
18	4A2 x 4A3	O
19	4B1 x 4B3	O
20	4B1 x 4B4	W
21	4B3 x 4B4	S
22	4C1 x 4C2	W
23	4C1 x 4C3	M
24	4C2 x 4C3	O
25	4D1 x 4D2	M
26	4D1 x 4D3	O
27	4D2 x 4D3	W
28	6A1 x 6A2	M
29	6A1 x 6A3	O
30	6A1 x 6A4	S
31	6A2 x 6A3	W
32	6A2 x 6A4	W
33	6A3 x 6A4	W
34	6B1 x 6B2	W
35	6B1 x 6B3	O
36	6B1 x 6B4	S
37	6B2 x 6B3	W
38	6B2 x 6B4	O
39	6B3 x 6B4	M
40	6C1 x 6C2	M
41	6C1 x 6C3	W
42	6C1 x 6C4	S
43	6C2 x 6C3	M
44	6C2 x 6C4	W
45	6C3 x 6C4	W
46	6D1 x 6D2	M
47	6D1 x 6D3	M
48	6D2 x 6D3	O
49	7A2 x 7A3	O
50	7A2 x 7A4	M
51	7A3 x 7A4	W
52	7B1 x 7B2	M
53	7B1 x 7B4	O
54	7B2 x 7B4	W
55	7C1 x 7C3	W
56	7C1 x 7C4	M
57	7C3 x 7C4	S
58	7D1 x 7D3	S
59	7D1 x 7D4	S
60	7D3 x 7D4	O
	WITHIN GROUP I	
61	2A2 x 2A3	W
62	2A2 x 2A4	M
63	2A3 x 2A4	W
64	2B1 x 2B3	S
65	2B1 x 2B4	O
66	2B3 x 2B4	M
67	2C1 x 2C2	W
68	2C1 x 2C3	S
69	2C1 x 2C4	O
70	2C2 x 2C3	O
71	2C2 x 2C4	S
72	2C3 x 2C4	M
73	2D1 x 2D3	S
74	2D1 x 2D4	O
75	2D3 x 2D4	W
76	6A1 x 6A2	M
77	6A1 x 6A4	O
78	6A2 x 6A4	S
79	6B2 x 6B3	O
80	6B2 x 6B4	M
81	6B3 x 6B4	W
82	6C2 x 6C3	O
83	6C2 x 6C4	M
84	6C3 x 6C4	S
85	6D1 x 6D2	S
86	6D1 x 6D3	M
87	6D2 x 6D3	M
88	7A2 x 7A3	O
89	7A2 x 7A4	S
90	7A3 x 7A4	M
91	7B1 x 7B2	M
92	7B1 x 7B3	O
93	7B2 x 7B3	W
94	7C1 x 7C2	M
95	7C1 x 7C3	O
96	7C2 x 7C3	O
97	7D2 x 7D3	O
98	7D2 x 7D4	O
99	7D3 x 7D4	S
	MONOSPORIDIAL LINES OF GROUP II	
100	2A2	O
101	2A4	O
102	2B2	O
103	2B3	O
104	2D2	O
105	2D3	O
106	4A1	O
107	4A2	O
108	4A3	O
109	4B1	M
110	4B3	O
111	4B4	O
112	4C1	O
113	4C2	O
114	4C3	O
115	4D1	O
116	4D2	O
117	4D3	O
118	6A2	O
119	6A3	O
120	6A4	O
121	6B2	O
122	6B3	O
123	6B4	O
124	6C2	O
125	6C3	O
126	6C4	O
127	7A3	O
128	7A4	O
129	7C1	S
130	7C4	O
	MONOSPORIDIAL LINES OF GROUP I	
131	2A2	O
132	2A4	O
133	2A3	O
134	2C1	O
135	2C2	O
136	2C3	O
137	6C4	O

* O = no chlorosis; W, weak; M, moderate; S, severe chlorosis.

a few days. Sori developed in the inflorescences of most but not all of the chlorotic plants. Different monosporidial combinations of *S. sorghi* probably have different degrees of pathogenicity that can be detected to some extent by the degree of chlorosis they cause although the writer's evidence is not entirely conclusive.

Inheritance of peridial color—Kulkarni (26) described two colors of peridial membrane in *Sphacelotheca sorghi*: one brownish, turning to dull gray; the other, shining gray, becoming pale gray on maturity. Melchers, Ficke, and Johnston (30) described the color of peridia produced by five physiologic races of *S. sorghi* on different varieties of sorghum. They concluded that races 1 and 4 were brown on all varieties, but races 2, 3, and 5 were brown on some and gray on others. From their work it would appear that peridial color is dependent on the host-physiologic race reaction. Tyler (46) obtained brown and gray peridial types on one variety of sorghum (Minnesota amber). In his experience the brown color seemed as fixed as the gray as there was no change of color when the brown and gray types were kept in the laboratory for a long time. Rodenhiser (36) stated that there were intergrading color types in inbred lines of *S. sorghi* as well as in crosses between *S. sorghi* and *S. cruenta.*

The writer obtained brown and gray types of *S. sorghi.* The brown collection,[5] number 22, is the inbred progeny of chlamydospore Texas A. The brownish-gray collection, 123, resulted from a cross between Texas $A3_1$ and 122B4. Sporidia were isolated from one chlamydospore, each, of collections 22 and 123 and attempts were made to inbreed the brown and gray collections. Two sporidia of opposite sex were selected on the basis of their reaction on Bauch's medium, cultured in potato-dextrose broth, and inoculated into five-week-old sorghum seedlings. Smutted heads obtained as a result of inoculating with two monosporidial lines from Collection 22 were designated as Collection 23, which is F_2 progeny of chlamydospore Texas A. Two sporidia from chlamydospore A of Collection 23 were again inoculated into sorghum seedlings, and the F_3 smutted heads resulting were numbered 24. In this manner the progeny of the brown type was continued up to the F_6 generation. The smutted heads produced from F_2 up to F_6 generations all had brown peridia. In figure 1 the Arabic numeral above the smutted head refers to the number of the generation or cross, while that on the side refers to the number of smutted heads obtained. In the same manner the

[5] For convenience, "collection" here is used to designate a particular lot of chlamydospores resulting from a particular cross.

Table 5. Cultural and Sexual Groups of Monosporidial Segregates from 28 Chlamydospores Resulting from Four Crosses Between Biotypes of *Sphacelotheca sorghi*

Number of chlamydospore	Number of sporidium*	Grouping† on the basis of: Color	Luster	Diameter of colony	Topography	Margin and edge	Tendency to sector	Sexual reaction‡
GROUP II								
2A (Brown)	1	A	A	A	A	A	A	A A
	2	B	A	A	B	B	A	B B
	3	A	A	A	A	A	A	C C
	4	B	A	A	B	B	A	C D
2B	2	A	A	A	A	A	A	A —
	3	B	A	A	B	B	A	B —
	4	C	A	A	A	A	A	B A
2C	1	A	A	A	A	A	A	A —
	2	B	A	A	B	A	A	B —
	3	C	A	A	B	A	A	A A
2D	2	A	A	A	A	A	A	A A
	3	A	A	A	A	A	A	B B
	4	B	A	A	B	B	A	A C
4A	1	A	A	A	A	A	A	A —
	2	A	B	B	B	B	A	B A
	3	A	B	C	B	B	A	B —
4B	1	A	A	A	A	A	A	A A
	3	B	B	B	B	B	B	A —
	4	C	A	A	C	A	A	B —
4C	1	A	A	A	A	A	A	A —
	2	B	B	B	B	B	B	B —
	3	B	B	B	B	B	B	B A
4D	1	A	A	A	A	A	A	A A
	2	B	A	A	A	A	A	B B
	3	A	A	A	A	A	A	A C
6A	1	A	A	A	A	A	A	A A
	2	B	A	B	A	B	A	B B
	3	B	B	B	B	B	A	A C
	4	C	A	C	A	B	B	C D
6B	1	A	A	A	A	A	A	A A
	2	A	B	A	B	B	A	B B
	3	A	B	A	B	A	A	A C
	4	B	B	B	A	A	A	B D
6C	1	A	A	A	A	A	A	A A
	2	B	A	A	B	B	A	B B
	3	C	B	A	B	B	A	C C
	4	D	A	B	A	C	B	D D
6D	1	A	A	A	A	A	A	A A
	2	A	A	B	A	A	A	B B
	3	A	A	B	A	A	A	B C
7A	2	A	A	A	A	A	A	A —
	3	B	A	A	B	B	A	A A
	4	A	A	B	B	A	A	B B
7B	1	A	A	A	A	A	A	A A
	2	B	A	A	A	B	B	B B
	4	C	A	B	B	B	B	A C

* The sporidia were numbered according to their position on the promycelium, number 1 being the sporidium at the tip of the promycelium.

† The groups into which the monosporidial lines fall are indicated by A, B, C, and D. It was necessary to determine the groups for each chlamydospore separately, so that the A group of chlamydospore IIA is not necessarily identical with the A group of another chlamydospore.

‡ The letters in the first column indicate the sexual groups obtained by pairing sporidial lines from the same promycelium, while those in the second column indicate the sexual groups obtained from pairing the same set of sporidial lines with lines obtained from another promycelium. The symbol — indicates no test.

Table 5 (Continued)

Number of chlamydospore	Number of sporidium*	Grouping† on the basis of						
		Color	Luster	Diameter of colony	Topography	Margin and edge	Tendency to sector	Sexual reaction‡
7C	1	A	A	A	A	A	A	A A
	3	B	A	B	B	B	B	B B
	4	C	A	B	B	B	B	C C
7D	1	A	A	A	A	A	A	A A
	3	A	A	B	B	B	B	B B
	4	A	A	B	B	B	B	B —
GROUP I								
2A (Gray)	1	A	A	A	A	A	A	A A
	2	B	A	B	A	A	A	B B
	3	B	A	B	A	A	A	C C
	4	B	A	B	A	A	A	A D
2B	1	A	A	A	A	A	A	A A
	3	B	A	A	B	A	A	B —
	4	B	A	A	B	A	A	A B
2C	1	A	A	A	A	A	A	A A
	2	A	A	A	A	A	A	B B
	3	B	A	B	A	A	B	B C
	4	B	A	B	A	A	B	A D
2D	1	A	A	A	A	A	A	A A
	3	B	A	A	A	A	A	B B
	4	B	A	A	A	A	B	A C
6A	1	A	A	A	A	A	A	A A
	2	B	A	B	B	A	A	B B
	4	C	B	C	C	A	A	A C
6B	1	A	A	A	A	A	A	A —
	2	B	A	B	B	A	A	B A
	3	A	A	B	B	A	A	B —
	4	A	A	A	C	A	A	A —
6C	2	A	A	A	A	A	A	A —
	3	B	A	B	B	A	A	A A
	4	A	A	A	C	A	A	B B
6D	1	A	A	A	A	A	A	A A
	2	A	A	B	A	A	A	B B
	3	B	A	A	B	A	A	C C
7A	2	A	A	A	A	A	A	A A
	3	B	A	A	B	A	A	A B
	4	C	A	A	B	A	A	B C
7B	1	A	A	A	A	A	A	A A
	2	B	A	A	A	A	A	B B
	3	A	A	A	A	A	A	A C
7C	1	A	A	A	A	A	A	A A
	2	B	A	B	B	A	A	B B
	3	B	A	B	B	A	A	C C
7D	2	A	A	A	A	A	A	A A
	3	B	B	A	A	A	B	B B
	4	C	B	A	B	A	B	C C

* The sporidia were numbered according to their position on the promycelium, number 1 being the sporidium at the tip of the promycelium.

† The groups into which the monosporidial lines fall are indicated by A, B, C, and D. It was necessary to determine the groups for each chlamydospore separately, so that the A group of chlamydospore IIA is not necessarily identical with the A group of another chlamydospore.

‡ The letters in the first column indicate the sexual groups obtained by pairing sporidial lines from the same promycelium, while those in the second column indicate the sexual groups obtained from pairing the same set of sporidial lines with lines obtained from another promycelium. The symbol — indicates no test.

Table 6. Segregation Ratios for Cultural Characters and Sex on Promycelia of the Chlamydospores Listed in Table 5 from Which Complete Sets of Sporidia Were Obtained

Chlamydospore number	Segregation ratios						
	Color	Size of colony	Luster	Topography	Margin	Tendency to sector	Sex
GROUP II (Brown)							
2A	2:2	4:0	4:0	2:2	2:2	4:0	1:2:1
6A	1:2:1	1:2:1	3:1	3:1	3:1	3:1	1:2:1
6B	3:1	3:1	3:1	3:1	3:1	4:0	2:2
6C	1:1:1:1	3:1	3:1	2:2	1:2:1	3:1	1:1:1:1
GROUP I (Gray)							
2A	1:3	3:1	4:0	4:0	4:0	4:0	1:2:1
2C	2:2	2:2	4:0	4:0	4:0	2:2	2:2
6B	3:1	2:2	4:0	1:2:1	4:0	4:0	2:2

progeny of brownish-gray Collection 123 was continued up to the F_4 generation.

In the F_2 generation (Cross 124) of the brownish-gray series, three distinct color types were obtained, viz., brown, brownish gray, and gray. (See Plate 3.) For F_3 progeny sporidia were isolated from a chlamydospore from the gray head of Collection 124 and inoculated into sorghum seedlings. Some of the F_3 smutted heads were brownish gray and some were gray. For the study of F_4 progeny, again a gray head of the F_3 was selected, and sporidia isolated from one promycelium of a chlamydospore from this head were inoculated into sorghum seedlings. Again some of the smutted heads were brownish gray and some were gray.

A later cross was made between lines from gray and brown collections to study the F_1 progeny. When sorghum seedlings were inoculated with this combination (21 A_1 x 124 A_3), eight smutted heads were produced, all of which were brownish gray. This cross was numbered 200. When two sporidia (200 A1 x 200 A3) from a chlamydospore 200A of this collection were inoculated into sorghum seedlings, 62 smutted heads were obtained. Of these, 3 were brown, 54 brownish gray, and 5 gray. Thus, the F_1 progeny of a brown x gray cross resulted in an intermediate color, brownish gray; and in the F_2 generation three types, brown, brownish gray, and gray, were obtained.

The results of the Texas A x Texas B series are hard to explain. Theoretically, a dicaryophyte should produce only one kind of sorus with one color only, barring mutation in the haploid lines or dicaryophytes. Nevertheless, three color types appeared in some of the crosses. This possibly may be due to (1) varietal impurity of the sorghum plants, i.e., differences in the host re-

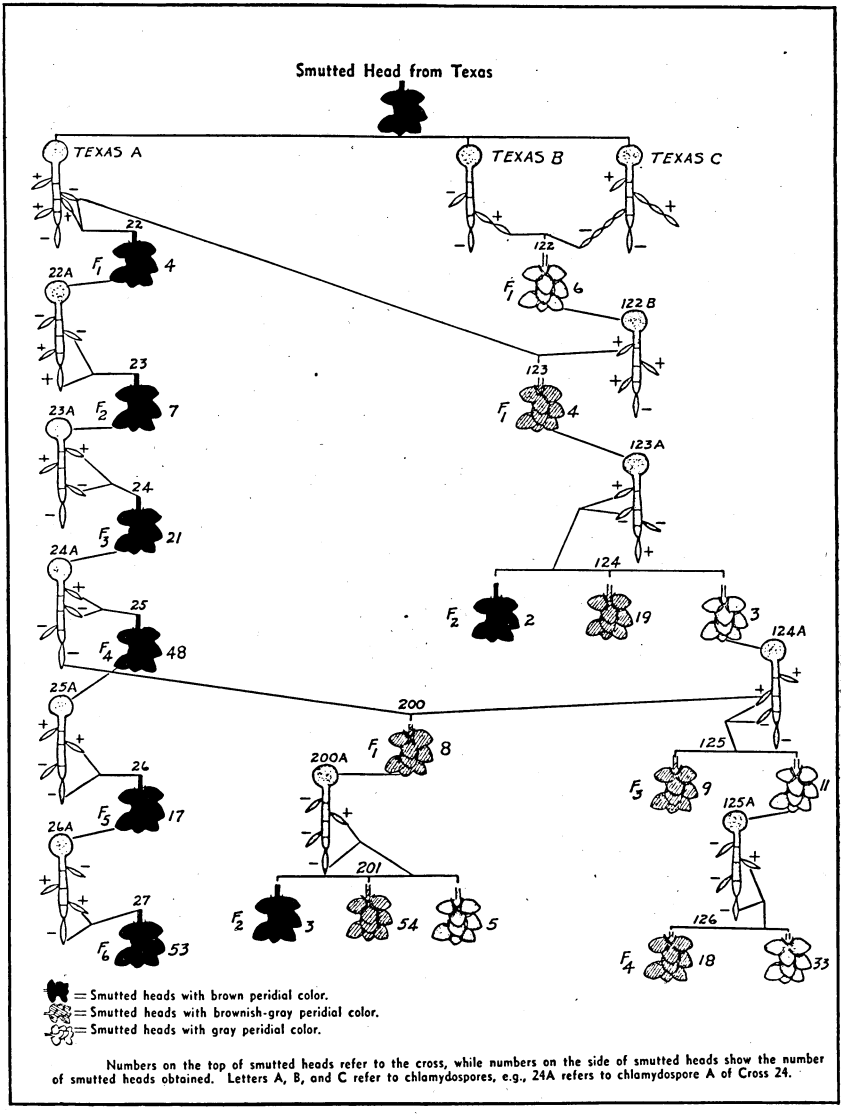

FIG. 1. SCHEMATIC REPRESENTATION OF THE INHERITANCE OF PERIDIAL COLOR CHARACTERISTICS IN *Sphacelotheca sorghi*

action; (2) mutation in the monosporidial lines with which inoculations were made; (3) mutation in the parasitic dicaryophase; or (4) delicately balanced factors for color in the dicaryophytes. The latter explanation seems most probable; otherwise some gray heads should have appeared also in the "brown x brown" crosses,

as mutations resulting in loss of factors for color are not common in the smuts.

In a study of five physiologic races of *Sphacelotheca sorghi,* races 1 and 4 produced smutted heads with brown peridia in the differential varieties, while races 2, 3, and 5 produced smutted heads with brownish-gray and gray peridia. This also agrees with previous results in which the gray collections tended to produce gray and brownish-gray head types.

Relation of Time of Planting to Severity of the Smut

Kulkarni (27) obtained no infection when he mixed inoculated sorghum seed with spores of *S. sorghi,* planted in pots and later transplanted in the field after incubation at 40° C. for four days. But there was as much as 57 per cent infection when the pots were incubated at 25° C. Reed and Faris (34) concluded that infection of susceptible varieties of sorghum may occur over a wide range of temperature, the optimum in their experiments being between 20° C. and 25° C. No infection occurred below 10° C. and above 37.5° C.

To ascertain whether the smut used in the present experiments differed from that used by others, chlamydospores from the gray head type of Collection 124 (Fig. 1) were used to inoculate seed of Minnesota amber sorghum, which was then planted in randomized triplicate rod rows in the field in the summers of 1935 and 1936. Seed was planted at weekly intervals from May 7 to June 4 in 1935 and from May 11 to June 8 in 1936. The soil temperature during the first two weeks in 1935 was far too low, i.e., the maximum temperature was around 63° F. and the minimum around 40° F.; hence the percentage of infection was low. But in the last three weeks, starting with May 21, the percentage of infection was higher because of the higher soil temperature. In 1936 the soil temperature was much higher than for comparable periods in the previous year, i.e., the maximum temperature was about 70° F. to 75° F. and the minimum 55° F. As there was relatively little variation in temperature during the entire planting period, there was fairly high infection throughout. These results agree with those of Reed and Faris (34) and indicate that fairly high percentage of infection occurs between 20° C. and 25° C., while no infection occurred below 10° C. The percentage of infection in 1935 was 6 per cent in the May 7 planting as compared with 18 per cent in the later plantings. These results indicate that if sorghum is planted early the percentage of smut infection is likely to be reduced to a certain extent.

Evidently the smut used by the writer did not differ greatly with respect to temperature requirements from that used by previous investigators.

A New Physiologic Race Resulting from an Intraspecific Cross

Potter (32) in 1915 demonstrated the distinct difference between covered and loose kernel smuts of sorghum by their behavior on several varieties of sorghum. Kulkarni (26) inoculated seeds of different strains of milo sorghum with chlamydospores of *Sphacelotheca sorghi* and *S. cruenta* and concluded that dwarf milo is susceptible to loose smut and resistant to covered smut. Potter and Melchers (33) inoculated different commercial varieties of *Sorghum vulgare* with spores of *S. sorghi* and pointed out that some of the varieties were highly resistant. Reed and Melchers (35) inoculated 250 varieties of sorghum with spores of *S. sorghi* and concluded that shallu, sorgos, broom corn, and kafirs were very susceptible, sudan grass moderately susceptible, and milo and feterita very resistant.

Tisdale, Melchers, and Clemmer (44) pointed out that milo, hegari, and feterita were attacked by kernel smut. They believed the smut on milo and hegari to be a distinct physiologic race, but they were not sure whether it was a race of *S. sorghi* or a hybrid between *S. sorghi* and *S. cruenta.* They also pointed out that the kernel smut on feterita was a distinct race of *S. sorghi.* Melchers, Ficke, and Johnston (29) designated these races as races 1, 2, and 3. They pointed out that Race 1 does not attack milo, hegari, and feterita; Race 2 attacks milo and hegari but not feterita; and Race 3 attacks feterita and feterita hybrids but not milo. They (30) later added two more physiologic races of *S. sorghi*, thus making five.

Rodenhiser (36) inoculated Reed kafir sorghum hypodermically with inter- and intraspecific crosses of *S. sorghi* and *S. cruenta.* He concluded that these two species hybridize readily and that the hybrid differs from the parents in infecting the host. Tyler (46) used F_1 chlamydospores from 10 crosses of known parentage for inoculating Minnesota amber seed by the dry method and with water suspension of spores. More infection resulted from inoculating with the dry spores, which he concluded was due either to heavier spore load or to the possibility that the spores suspended in water germinated too rapidly and failed to cause infection. He noticed differences in parasitic capabilities of different chlamydospore collections and concluded

Table 7. Percentage of Infection on 48 Varieties and Strains of Sorghum Inoculated with Chlamydospores of Race 6 from Gray Head Type Resulting from Cross 124 of *Sphacelotheca sorghi* in the Field at University Farm, St. Paul, Minnesota, 1935 and 1936

Name of variety selection or hybrid	Accession number*	Per cent smut†		
		1935	1936	Average
Beaver	C.E. 2534	0	0	0
Day milo	C.E. 2536	0	0	0
Darso	C.E. 2521	0	0	0
Dwarf Yellow milo	C.E. 2523	0	0	0
Dwarf Yellow milo x Early White milo	C.E. 2524	0	0	0
Dwarf Yellow milo x Early White milo	C.E. 2925	0	0	0
Dwarf Yellow milo x Early White milo	C.E. 2540	0	0	0
Dwarf Yellow milo x Early White milo	C.E. 2541	0	0	0
Dwarf Yellow milo x Early White milo	C.E. 2542	0	0	0
Extra dwarf milo	T.S. 13352	0	0	0
Feterita	C.E. 2522	0	0	0
White milo		0	0	0
Wheatland	C.E. 2531	1	1	1
Ajax		4	4	4
H. C. Selection 336	C.E. 2550	6	2	4
H. C. No. 312		3	8	5.5
Hegari	K.B. 2518	6	6	6
Sudan grass		4	9	6.5
Kaoliang		13	1	7
Greenley	C.E. 2527	8	15	11.5
Dwarf Yellow milo	C.E. 2524	11	15	13
Buff Durra		26	3	14.5
Broom corn		30	16	23
Maldani		22	25	23.5
Freed		43	17	30
Red Amber		38	23	30.5
Blackhull Kafir	C.E. 2519	29	48	38.5
Cheyenne	C.E. 2545	42	35	38.5
Minnesota Amber		39	40	39.5
Club Kafir	C.E. 2517	27	55	41
Modoc	C.E. 2543	50	39	44.5
Kansas Orange	C.E. 2549	51	40	45.5
Leoti Red	C.E. 2547	55	36	45.5
Kalo	C.E. 2525	47	53	50
Red Kafir	C.E. 2515	45	55	50
Early Sumac	C.E. 2546	64	38	51
Early Kalo	C.E. 2526	40	67	53.5
Dawn Selection Kafir	C.E. 2431	58	53	55.5
Blackhull Kafir	C.E. 2518	49	63	56
Red Kafir	C.E. 2436	53	59	56
Shallu		38	79	58.5
Tricher	C.E. 2544	66	55	60.5
Shrock		69	53	61
Dwarf Freed		55	69	62
Atlas	C.E. 2548	53	72	62.5
Weskan Kafir	C.E. 2520	57	79	68
Reed Kafir	C.I. 628	80	80	80
Pink Kafir	C.E. 2514	73	92	82.5

* C.E. refers to cooperative experiment numbers of Kansas State Agricultural college and Division of Cereal Crops and Diseases, U. S. Department of Agriculture.
K.B. refers to "Kansas Botany" number of Kansas State college.
T.S. refers to Texas State Agricultural College numbers.
C.I. refers to accession number of the Division of Cereal Crops and Diseases, U. S. Department of Agriculture.

† Most percentages are based on counts of over 100 heads; in a few instances between 50 and 100 were counted.

that close inbreeding and outbreeding of primary, secondary, and tertiary monosporidial lines from chlamydospores of one collection could result in chlamydospore lines that would produce uniformly high or low percentages of infection.

The writer inoculated 48 varieties of sorghum[6] with chlamydospores from gray smutted heads of Collection 124 (Fig. 1) and planted them in triplicate rod rows in the field in the summer of 1935. Infection occurred in primary as well as secondary heads of many varieties. This experiment was continued in the summer of 1936. Table 7 gives the results of all the varieties tried for the two years. From the results it appears that Collection 124 differs from all the five physiologic races of *S. sorghi* so far known.

Seed of differential varieties of sorghum were inoculated with spores of Collection 124 and planted in triplicate rows in the field in the summer of 1936. The five physiologic races[7] of *S. sorghi*[8] were also tried in triplicate rows in the field. The results showed that Collection 124 differs from the hitherto known five physiologic races of *S. sorghi.* The writer has designated it Race 6 and named it the hegari-kafir race since it attacks hegari moderately and kafirs severely. As the essential details have been published (50), they will not be repeated here. The writer has added hegari to the list of seven differential hosts. While this new race is not known to occur in nature, it was felt desirable to add it to the key prepared by Melchers, Ficke, and Johnston (30) in order to show its relation to the five races previously described.

Key for the identification of physiologic races of *Sphacelotheca sorghi*

A. Kafir x feterita (H.C. 2423), resistant.
  B. Dwarf yellow milo (C.I. 332), highly resistant.
    C. White yolo (K.B. 2525), resistant.
      D. Hegari (K.B. 2518), resistant ..... Race 1
      D.D. Hegari (K.B. 2518), susceptible ..... Race 6
    C.C. White yolo (K.B. 2525), susceptible ..... Race 4
  B.B. Dwarf yellow milo (C.I. 332), moderately susceptible ..... Race 2

[6] The writer wishes to acknowledge his thanks to the following people for the supply of seed of different varieties of sorghum: Dr. J. H. Parker, Agronomist in charge of Small Grain and Sorghum Breeding, Kansas State College, Manhattan, Kansas; Prof. L. E. Melchers, Professor of Plant Pathology, Kansas State College, Manhattan, Kansas; and Dr. J. F. Martin, Senior Agronomist, in charge of Sorghum and Broom Corn Investigation, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D.C.

[7] These had been called physiologic forms previously, but in accordance with the resolution passed by the Sixth International Botanical Congress in September, 1935, the term "physiologic race" is substituted for "physiologic form."

[8] The writer wishes to acknowledge his thanks to Prof. L. E. Melchers, Kansas State College, Manhattan, Kansas, for the supply of smutted heads of five physiologic races of *S. sorghi.*

A.A. Kafir x feterita (H.C. 2423), susceptible.
B. Pierce kaferita (K.B. 2547), feterita x kafir (F.C.I. 8917), and feterita (S.P.I. 51989), highly resistant.....Race 5
B.B. Pierce kaferita (K.B. 2547), feterita x kafir (F.C.I. 8917), and feterita (S.P.I. 51989), susceptible..Race 3

Obviously new physiologic races of *S. sorghi* can arise by hybridization between biotypes within the species even if isolated from promycelia of chlamydospores from a single smut sorus. Furthermore, the one which the writer produced differs decidedly from the hitherto known five physiologic races, and it seems probable that similar events can transpire in nature.

Effect of Different Seed Treatments on the Control of *Sphacelotheca sorghi*

Freeman and Umberger (15) as early as 1908 advocated treatment of sorghum seed against covered kernel smut (grain smut) with formalin solution, hot water, and copper sulphate. Kulkarni (26) inoculated sorghum seed with chlamydospores of *S. sorghi* and dipped them for 10 minutes in various strengths of copper sulphate (½ per cent, 1 per cent, and 2 per cent). He obtained 100 per cent control in treated seed lots, while the check plot had 20 per cent infection. Johnston and Melchers (22) made extensive studies on the treatment of sorghum to control covered kernel smut. They used several dip and dust treatments, and concluded that (1) copper carbonate, dehydrated copper sulphate, Dosch copper-lime, flowers of sulphur and the finer makes of sulphur dusts, Corona 40 S, Corona 640, and Coppercarb are almost as effective as the standard formaldehyde treatment; (2) the dust treatments all cause less seed injury than formaldehyde treatments; and (3) copper carbonate and certain sulphur dusts seem to be the most promising from the standpoint of ease and rapidity of application, cheapness, smut control, and freedom from seed injury.

The writer used several dip and dust treatments, including New Improved Semesan Jr., New Improved Ceresan, and several forms of sulphur. Seeds of the variety, Minnesota amber, were mixed with chlamydospores of *S. sorghi*, treated with different fungicides, and planted in triplicate rod rows in the field in a randomized block. These trials were made in the summers of 1935 and 1936 at University Farm, St. Paul, Minnesota. Details are not given, but New Improved Ceresan, one half ounce per bushel, eliminated smut in both years. Ceresan, Koppers "Flota-

tion" sulphur, wettable sulphur, powdered flowers of sulphur, gas sulphur, and Kolo dust also gave effective control, ranging from 98.4 to 99.5 per cent when the average amount of smut in the checks was 38 per cent. There was no evidence of any seed injury by any of the fungicides.

Summary of Data Obtained Regarding *Sphacelotheca sorghi*

The primary object of the investigation was to find out as much as possible about the genetics of certain sorghum smuts, especially *S. sorghi*. It is evident that there are many haploid biotypes within the species that can be distinguished readily by cultural characters; that some lines mutate; that there are many sex groups, as determined by the Bauch test, by sporidial fusions, by the chlorosis caused in sorghum plants inoculated with pairs of compatible lines, and, finally, by the production of chlamydospores in plants inoculated with compatible pairs of lines. There is evidence also that recombinations of factors for many characters are extremely common as a result of sexual fusions and subsequent segregation when gametic sporidia are formed on the promycelia. The species, then, comprises a very large number of fairly freely interbreeding biotypes; consequently there is a wide range of characters, such as pathogenicity and peridial color in smutted heads. The fact that a distinct physiologic race resulted from a series of crosses between haploid lines shows definitely that the pathogenicity of the species may vary. It seemed desirable to learn, therefore, whether new combinations could result also from hybridization between another species of *Sphacelotheca* (*S. cruenta*) and *Sorosporium reilianum*.

Hybridization Between *Sphacelotheca cruenta* and *Sorosporium reilianum*

Criteria for Determining Sexual Compatibilities

The Bauch test proved reliable for determining sex groups in *Sphacelotheca sorghi* as shown by Tyler and confirmed by the writer. Rodenhiser (37) concluded that this test also is a reliable criterion of compatibility between intraspecific and interspecific lines of *S. sorghi* and *S. cruenta*. The writer confirmed Rodenhiser's conclusions but found (48) that the test is not reliable for *Sorosporium reilianum* or in combinations with *S. sorghi* and *S. cruenta*.

Sporidial and hyphal fusions have been studied in various smut fungi by several investigators (11, 20, 25, 39). In the sorghum smuts Tyler (45) and Isenbeck (21) observed sporidial fusion in *Sphacelotheca sorghi* and Rodenhiser (36) in *S. sorghi* and *S. cruenta.* Shumway (38) failed to demonstrate sporidial fusion in *Sorosporium reilianum.* The writer, too, observed sporidial fusion between sexually compatible lines of *S. sorghi* and *S. cruenta* on slightly alkaline malt agar (3 per cent malt extract and 2 per cent agar) and slightly alkaline nonnutrient agar (2 per cent agar), but sporidial fusions could not be seen in monosporidial lines of *Sorosporium reilianum* and between lines of *Sphacelotheca cruenta* and *Sorosporium reilianum.*

Rodenhiser (36) pointed out that chlorosis on the leaves of sorghum plants inoculated with paired lines of *Sphacelotheca sorghi* and *S. cruenta* could be taken as an index of sex compatibility. Tyler and Shumway (47) obtained similar results with sexually compatible lines of *S. sorghi* and *Sorosporium reilianum.* The writer obtained different degrees of chlorosis (i.e., severe, moderate, and weak) on leaves of sorghum plants inoculated with sexually compatible intraspecific lines of *Sphacelotheca sorghi, S. cruenta,* and *Sorosporium reilianum* as well as with intergeneric combinations of lines of *Sphacelotheca sorghi* x *Sorosporium reilianum* and *Sphacelotheca cruenta* x *Sorosporium reilianum.* The same was true on sorghum leaves inoculated with sexually compatible f_1 lines of the intergeneric hybrid between *Sphacelotheca sorghi* and *Sorosporium reilianum* (48) and between *Sphacelotheca cruenta* and *Sorosporium reilianum.* This shows that chlorosis can be taken as an index of sex compatibility between monosporidial lines of two genera of sorghum smuts or of the intergeneric hybrids.

Relative Ease of Hybridization

Rodenhiser (36) crossed *Sphacelotheca sorghi* and *S. cruenta* and concluded that they hybridize readily and that hybrids between them are found in nature. Tyler and Shumway (47) crossed *S. sorghi* and *Sorosporium reilianum* and pointed out that the hybrid was intermediate between the parents with respect to sori and size of chlamydospores. The writer (48) made more crosses between these two genera and noticed that they cross readily and produce fertile hybrids because certain combinations of f_1 lines caused infection on sorghum plants.

When the writer hypodermically inoculated five haploid lines of *Sphacelotheca cruenta* and seven of *Sorosporium reilianum* singly and in paired combinations into sorghum seedlings, 11 out of 18 paired combinations produced chlorosis on leaves in 8 to 12 days, but none of the single lines caused infection. At maturity normal sori developed in the inflorescences of the chlorotic plants. Different combinations of monosporidial lines of the two genera produced sori differing in size and shape (49). Apparently, then, these two genera hybridize readily, as almost all of the compatible combinations produced sori containing viable chlamydospores as did also certain combinations of f_1 lines.

Characters of the F_1 Generation of *Sphacelotheca cruenta* x *Sorosporium reilianum*

Sori—Different monosporidial combinations of *Sphacelotheca cruenta* x *Sorosporium reilianum* produced sori differing in shape and size. Certain combinations produced sori resembling those caused by *S. cruenta*, e.g., Cross 303 (cA2 x rC2)[9] and some produced sori like those of *S. cruenta*, infecting individual ovaries. Crosses 304 (cA1 x rA3) and 305 (cA1 x rA4) produced sori which resembled to some extent the sori of *Sorosporium reilianum*, infecting the entire inflorescence. Certain combinations of monosporidial lines of the two genera, however, produced sori resembling in shape those of long smut of sorghum caused by *Tolyposporium filiferum*, i.e., crosses 302 (cA2 x rB1), 300 (cA2 x rB2), 306 (cB2 x rA3), and 307 (cB3 x rA3). Some of the monosporidial combinations of these two genera produced sori which were very long, narrow, and sharply pointed and thus differed markedly from *Sphacelotheca cruenta*, *Sorosporium reilianum*, and *Tolyposporium filiferum*. Cross 300 (cA2 x rB2) produced long, narrow sori measuring about 3 cm. Cross 301 (cA1 x rB2) produced long, narrow, and pointed sori, measuring about 7 cm. (Plate 4 and Fig. 2.)

Chlamydospores—Hanna and Popp (19) and Holton (20) in hybridizing *Ustilago avenae* and *U. levis* noticed that the chlamydospores of the hybrid were echinulate. Flor (14) found that hybrid chlamydospores of *Tilletia tritici* x *T. levis* were smooth. Allison (1) obtained echinulate chlamydospores in the F_1 generation in crosses between *U. hordei* and *U. medians*.

[9] For convenience, "c" and "r" are placed before the capital letters to indicate "cruenta" and "reilianum." Thus cA2 means the sporidium (or sporidial line) from the second cell of the promycelium of chlamydospore A of *S. cruenta;* rC2, similarly, refers to sporidium number 2 from the promycelium of chlamydospore C of *S. reilianum.*

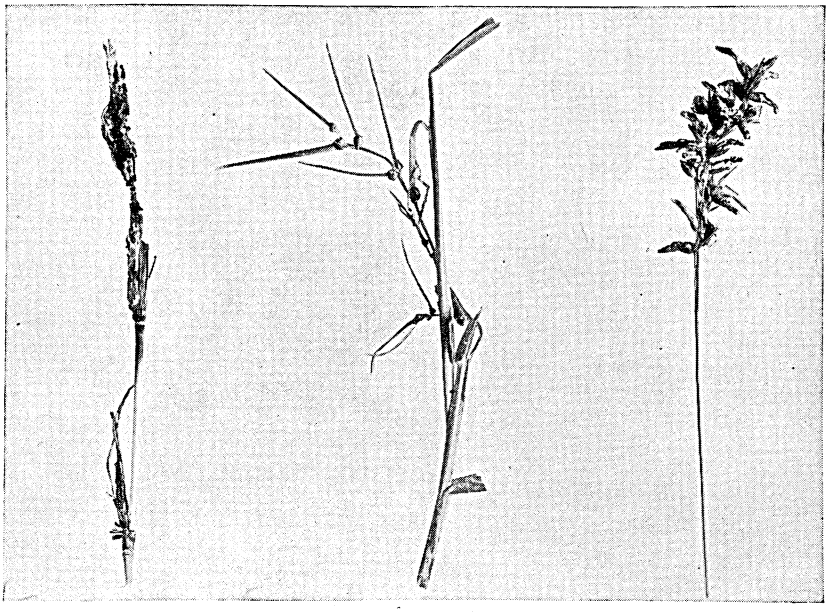

FIG. 2. PANICLES OF SORGHUM SMUTTED BY (*Sphacelotheca cruenta* x *Sorosporium reilianum*), SHOWING THREE TYPES, VIZ., THAT ON THE LEFT RESEMBLING *Sorosporium reilianum*, THAT ON THE RIGHT RESEMBLING *Sphacelotheca cruenta*, AND THAT IN THE CENTER BEING OF THE LONG TYPE

Tyler and Shumway (47) noticed that echinulation of chlamydospores of the hybrid between *Sphacelotheca sorghi* and *Sorosporium reilianum* was intermediate. The writer (48) in a more detailed study of this hybrid found that the hybrid chlamydospores were echinulate like those of *S. reilianum.* The chlamydospores of *S. reilianum* and the hybrid were not significantly different in diameter, but were significantly larger than those of *Sphacelotheca sorghi.*

When 100 chlamydospores each of *Sphacelotheca cruenta, Sorosporium reilianum,* and the hybrid between them were measured it was found that the average diameters were 5.94, 11.45, and 8.81μ, respectively. The hybrid chlamydospores, therefore, were intermediate in size between those of the parents (Table 8 and Fig. 3).

Effect of temperature on the germination of chlamydospores—When the writer tried the germination of spores of *Sphacelotheca cruenta, Sorosporium reilianum,* and the hybrid in 2 per cent sucrose solution at various temperatures (Table 9), the minimum temperature for all of them is 10° C., while the maximum for

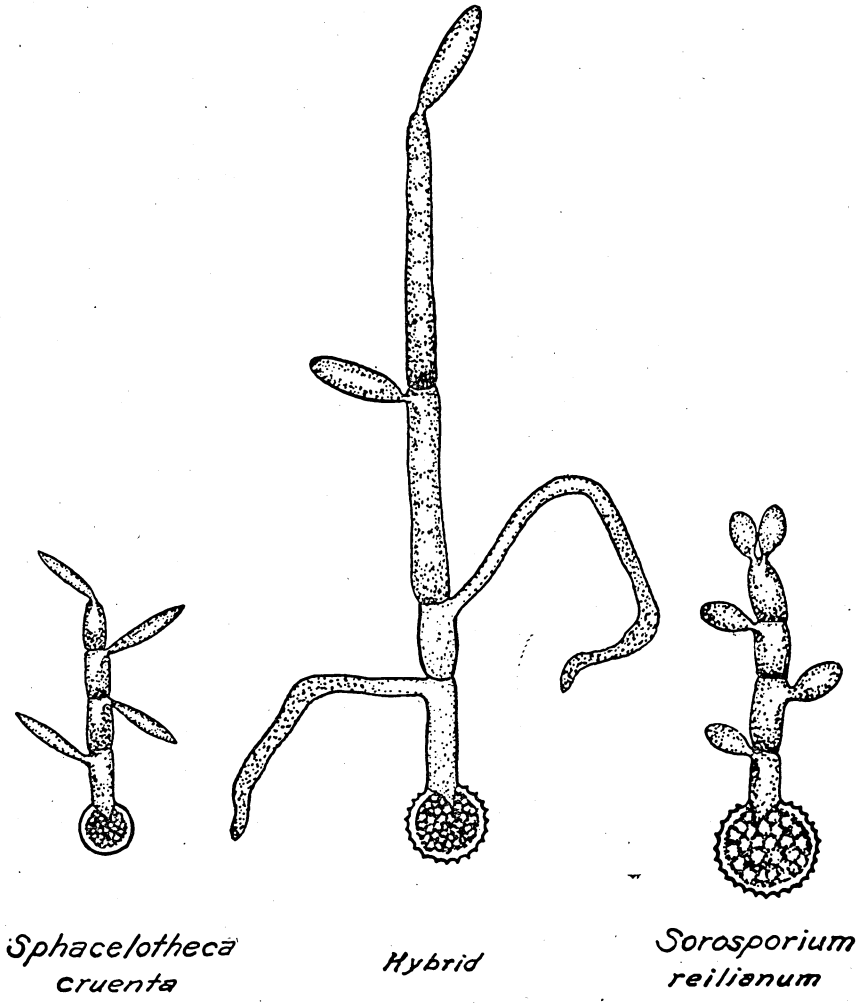

FIG. 3. THE CHLAMYDOSPORES, PROMYCELIA, AND SPORIDIA OF *Sphacelotheca cruenta*, *Sorosporium reilianum*, AND THE HYBRID (*Sphacelotheca cruenta* x *Sorosporium reilianum*)

Sphacelotheca cruenta is 35° C., for *Sorosporium reilianum* 40° C., and for the hybrid 45° C. The optimum for *Sphacelotheca cruenta* is about 25° C., for *Sorosporium reilianum* about 27° C., and for the hybrid 30° C. The hybrid thus has a higher optimum than either of the parents, and a higher percentage of spores germinate at higher temperatures than is true of the parents (Table 9). Kamat (23) showed that the optimum temperature for *Tolyposporium filiferum* lies between 28° C. and 33° C. He obtained as

Table 8. Diameters, in Microns, of Chlamydospores and Lengths of Sporidia and Promycelia of *Sphacelotheca cruenta*, *Sorosporium reilianum*, and the Hybrid (*Sphacelotheca cruenta* x *Sorosporium reilianum*)

	Sphacelotheca cruenta	*Sorosporium reilianum*	Hybrid	Difference required for significance
Chlamydospores	5.94	11.45	8.81	0.15
Sporidia	11.26	5.64	12.70	0.24
Promycelia	25.21	27.38	79.00	3.94

high as 91 per cent germination at 28° C. and 88 per cent at 33° C., while at 39° C. 8 per cent germinated on 1 per cent potato-dextrose agar. The percentages of germination in distilled water were proportionately lower.

This intergeneric hybrid, therefore, resembles *Tolyposporium filiferum* in respect to temperature relations for the germination of chlamydospores.

Promycelia—When the writer studied the germination of chlamydospores of the hybrid in distilled water, 2 per cent sucrose solution, and one per cent potato-dextrose-agar drops, he noticed that promycelia produced by a great majority of the germinating chlamydospores were very long, although some were as short as 20μ and some were 25 to 27μ, like those of *Sphacelotheca cruenta* and *Sorosporium reilianum*. Some, however, were as long as 125μ. The average length of the promycelium (average of 50 promycelia measured) is 79μ and is significantly larger than those of the parents (Table 8 and Fig. 3). The value of "F" exceeds the 1 per cent point (40).

The promycelia bear either sporidia or hyphal branches or both. In some cases two sporidia (one on each segment) and two hyphal branches (one on each segment) were seen. In other

Table 9. Percentage of Germination of Chlamydospores of *Sphacelotheca cruenta*, *Sorosporium reilianum*, and of the F_1 Hybrid Chlamydospores in 2 Per Cent Sucrose Solution

Temperature in degrees C.	Percentage germination		
	Sphacelotheca cruenta	*Sorosporium reilianum*	Hybrid
5	0	0	0
10	21	1	8
15	48	8	14
20	60	25	22
25	69	50	32
27	67	54	47
30	52	49	60
35	27	16	49
40	0	4	15
45	0	0	7
50	0	0	.....

cases four sporidia or four hyphal branches (one on each segment) were observed.

The length of the hyphal branches varied from 18μ to about 70μ. Attempts to grow the hyphal branches cut from the promycelium failed.

Sporidia (f_1)—Most of the sporidia produced on the hybrid promycelia were longer than those of *Sphacelotheca cruenta*, although some were only as long as those of *S. cruenta* and some as short as those of *Sorosporium reilianum*. The average of 100 sporidia of *Sphacelotheca cruenta* was 11.26μ, of *Sorosporium reilianum* 5.64μ, and of the hybrid 12.7μ. The sporidia of the hybrid are significantly larger than those of either parent, the value of "F" exceeding the 1 per cent point (40) (Table 8 and Fig. 3).

Segregation of factors for cultural characters—Twenty-four monosporidial lines isolated from six chlamydospores from Cross 300 (cA2 x rB2) were studied for their cultural characters (Table 12). Nine out of 24 f_1 lines were mycelial and 15 sporidial, while the parental lines, cA2 and rB2, were sporidial. The f_1 lines differed from the parental lines in topography and luster also, i.e., the parental lines were almost flat and shiny while the f_1 lines were mostly raised, convex, or pulvinate and dull in luster. These 24 f_1 lines of the hybrid fall into six distinct cultural types (Table 12 and Plate 5). Table 13 shows the segregation ratios of factors for cultural characters and sex. The segregation ratios for cultural characters on individual promycelia are 4:0, 3:1, 2:2, 1:2:1, and 1:1:1:1. The segregation of factors for cultural characters is independent of those for sex.

Segregation of factors for sex—Twenty-four monosporidial lines isolated from six chlamydospores from Cross 300 (cA2 x rB2) also were studied for segregation of factors for sex. Lines which were sexually compatible produced chlorosis on the leaves of sorghum plants when inoculated hypodermically although different monosporidial combinations produced different degrees of chlorosis (Table 15). Chlorotic plants later produced sori containing chlamydospores. The segregation of factors for sex is recorded in table 10. The sporidia on promycelia of chlamydospores A, B, and F fall into two sex groups (ratio 2:2), while the sporidia on chlamydospores C and D fall in three sex groups (ratio 1:2:1) and the sporidia on chlamydospore E fall into four sex groups (ratio 1:1:1:1).

When sporidia from two chlamydospores, A and E, were paired in all possible combinations and inoculated into sorghum seed-

Table 10. Segregation of Factors for Sex as Determined by Pairing the f_1 Lines from Promycelia of Six Chlamydospores Resulting from Cross 300 (cA2 x rB2), *Sphacelotheca cruenta* **x** *Sorosporium reilianum*

A

	1	2	3	4	Sexual group
1	−	−	+	+	1
2	−	−	+	+	1
3	+	+	−	−	2
4	+	+	−	−	2

B

	1	2	3	4	Sexual group
1	−	−	+	+	1
2	−	−	+	+	1
3	+	+	−	−	2
4	+	+	−	−	2

C

	1	2	3	4	Sexual group
1	−	+	+	+	1
2	+	−	+	+	2
3	+	+	−	−	3
4	+	+	−	−	3

D

	1	2	3	4	Sexual group
1	−	+	+	+	1
2	+	−	+	+	2
3	+	+	−	−	3
4	+	+	−	−	3

E

	1	2	3	4	Sexual group
1	−	+	−	+	1
2	+	−	−	+	2
3	−	−	−	+	3
4	+	+	+	−	4

F

	1	2	3	4	Sexual group
1	−	−	+	+	1
2	−	−	+	+	1
3	+	+	−	−	2
4	+	+	−	−	2

lings, the writer obtained evidence of seven sex groups on the basis of reactions on sorghum plants (Table 11). There seem to be multiple factors for sex in the f_1 lines of the hybrid as is true also of *Ustilago zeae*, *Sphacelotheca sorghi*, and several other smuts.

Variation in haploid lines—Cultural variants, originating as sectors or patches in cultures of various fungi, have been designated as variants or "mutants." In *Ustilago zeae*, Stakman et al. (42, 43) have shown that an indefinite number of culturally dis-

Table 11. The Result of All Possible Pairings Between Eight f_1 Lines Isolated from Promycelia of Two Chlamydospores of Cross 300 (cA2 x rB2), *Sphacelotheca cruenta* x *Sorosporium reilianum*

		A				E				
		1	2	3	4	1	2	3	4	Sexual group
A	1	−	−	+	+	−	+	−	−	1
	2	−	−	+	+	+	−	−	−	2
	3	+	+	−	−	−	+	+	+	3
	4	+	+	−	−	−	+	+	+	3
E	1	−	+	−	−	−	+	−	+	4
	2	+	−	+	+	+	−	−	+	5
	3	−	−	+	+	−	−	−	+	6
	4	−	−	+	+	+	+	+	−	7

tinct lines could be obtained by transferring from sectors that appeared from certain monosporidial lines. Hanna (18) observed mutants in monosporidial cultures of *Sorosporium reilianum*, and Rodenhiser (37) noticed that four of 90 f_1 lines of the hybrid between *Sphacelotheca sorghi* and *S. cruenta* mutated frequently when cultured on 2 per cent potato-dextrose agar.

In studying the cultural characters of 24 f_1 lines isolated from six chlamydospores of Cross 300 on potato-dextrose agar, the writer observed several sectors which were culturally different from the parental lines. Furthermore, when cultures of four f_1 lines of the hybrid (from chlamydospore D) were compared with parental lines cA2 and rB2 on five media, a few sectors developed on certain media (Table 14). All the lines, parental as well as f_1, grew very slowly on Medium 1 but fairly well on all other media, except for line cA2 *(Sphacelotheca cruenta)* which grew very slowly on Medium 2. Parental line cA2 *(S. cruenta)* did not sector on any medium, while parental line rB2 *(Sorosporium reilianum)* sectored on media 2, 3, and 4. The f_1 line D3 sectored on Medium 2, while the f_1 line D4 sectored on Medium 4. On Medium 2, which contains 0.012 per cent ammonium phosphate, all four f_1 lines grew better than either of the parental lines. When transfers were made from three f_1 lines (one sector from each line) and cultured in Erlenmeyer flasks on 1.5 per cent potato-dextrose agar these variants were distinct from the f_1 lines. The sector

Table 12. Grouping for Cultural Characters and Sex of f_1 Lines Isolated from Promycelia of Six Chlamydospores of Cross 300 (cA2 x rB2) Between *Sphacelotheca cruenta* and *Sorosporium reilianum*

Chlamydospores	Sporidium number*	Characters and Groups†							
		Size of colony	Color	Growth type	Luster	Topography	Margin and edge	Tendency to sector	Sex‡
A	1	A	A	A	A	A	A	A	A A
	2	B	B	B	A	B	B	B	A B
	3	A	A	A	A	A	A	A	B C
	4	C	A	A	A	C	A	B	B C
B	1	A	A	A	A	A	A	A	A —
	2	A	B	B	A	A	B	B	A —
	3	B	B	A	A	B	C	A	B —
	4	C	A	B	A	C	D	B	B —
C	1	A	A	A	A	A	A	A	A —
	2	B	B	A	A	B	B	B	B —
	3	B	B	A	A	B	B	A	C —
	4	B	B	A	A	B	B	B	C —
D	1	A	A	A	A	A	A	A	A —
	2	B	B	A	A	A	A	A	B —
	3	A	C	B	A	B	A	B	C —
	4	B	D	B	A	B	B	A	C —
E	1	A	A	A	A	A	A	A	A A
	2	A	B	B	A	A	A	B	B B
	3	B	C	A	A	B	B	B	C C
	4	A	B	B	A	A	A	A	D D
F	1	A	A	A	A	A	A	A	A —
	2	B	B	B	A	B	A	B	A —
	3	C	A	A	A	C	B	B	B —
	4	D	C	A	A	A	B	A	B —

* The sporidia are numbered according to their position on the promycelium, number 1 being the sporidium at the tip of the promycelium.

† The groups into which the monosporidial lines fall are indicated by A, B, C, and D. It was necessary to determine the groups for each chlamydospore separately, so that the A group of chlamydospores HI is not necessarily identical with the A group of another chlamydospore.

‡ The letters in the first column indicate the sexual groups based on pairing sporidial lines from the same promycelium, while those in the second column indicate sexual groups on the basis of pairing them with sporidial lines obtained from another promycelium. The symbol — indicates no test.

Table 13. Segregation Ratios for Cultural Characters and Sex of f_1 Lines Isolated from Promycelia of Six Chlamydospores of Cross 300 (cA2 x rB2), *Sphacelotheca cruenta* x *Sorosporium reilianum*, on the Basis of Data Given in Table 12

Chlamydospore number	Cultural characters							Sex
	Size of colony	Color	Growth type	Luster	Topography	Margin and edge	Tendency to sector	
A	1:2:1	3:1	3:1	4:0	1:2:1	3:1	2:2	2:2
B	1:2:1	2:2	2:2	4:0	1:2:1	1:1:1:1	2:2	2:2
C	3:1	3:1	4:0	4:0	3:1	3:1	2:2	1:2:1
D	2:2	1:1:1:1	2:2	4:0	2:2	3:1	3:1	1:2:1
E	3:1	1:2:1	2:2	4:0	3:1	3:1	2:2	1:1:1:1
F	1:1:1:1	1:2:1	3:1	4:0	1:2:1	2:2	2:2	2:2

Table 14. Size of Colonies and Number of Sectors in Parental Lines cA2 *(Sphacelotheca cruenta)* and rB2 *(Sorosporium reilianum)* and Four f_1 Lines from the F_1 Hybrid Chlamydospore 300D, on Five Different Media

f_1 Line	Medium*				
	1	2	3	4	5
	Diameter of colonies in mm.				
cA2	4	10	60	55	48
rB2	8	25	35	30	46
300D1	8	44	44	36	38
300D2	10	36	40	34	44
300D3	10	32	42	52	35
300D4	9	35	46	60	50
	Number of sectors				
cA2	0	0	0	0	0
rB2	0	2	1	1	0
300D1	0	0	0	0	0
300D2	0	0	0	0	0
300D3	0	1	0	0	0
300D4	0	0	0	4	0

* 1. Agar 1.5 per cent.
2. Sucrose 3 per cent, agar 1.8 per cent, ammonium phosphate 0.012 per cent.
3. Peptone 1.5 per cent, dextrose 1.5 per cent, agar 1.5 per cent.
4. Malt extract 2 per cent, dextrose 1 per cent, agar 1.5 per cent.
5. Potato-dextrose agar 1.5 per cent.

isolated from f_1 line E3 was cinnamon in color and sporidial, while f_1 line E3 was white and mycelial. Similarly, f_1 line C4 was mycelial and chocolate in color with a gray center, while the variant from it was sporidial and gray in color. Line F3, which was gray and mycelial, produced a black sporidial sector (Plate 6).

Pathogenicity of f_1 Lines

Four monosporidial lines of each of six chlamydospores of Cross 300 (cA2 x rB2) were combined in all possible combinations and inoculated hypodermically into sorghum seedlings. Eight to 12 days after inoculation certain sexually compatible combinations produced severe chlorosis on leaves, others produced moderate chlorosis, and still other combinations produced weak chlorosis (Table 15). The different degrees of chlorosis caused by different monosporidial combinations of the f_1 lines may indicate different degrees of pathogenicity although more work should be done to determine how reliable an index of pathogenicity chlorosis may be. Chlorotic plants produced sori on maturity. Different monosporidial combinations of the f_1 lines produced sori differing in size and shape. Some of the F_2 sori resembled those of *Sphacelotheca cruenta* and some resembled those of *Sorosporium reili-*

Table 15. The Degree of Chlorosis Resulting from Inoculating Sorghum Seedlings Hypodermically with Various Combinations of f_1 Lines from Cross 300 (cA2 x rB2), *Sphacelotheca cruenta* x *Sorosporium reilianum*

Combinations of f_1 lines and degree of chlorosis*					
A1 x A2	O	C1 x C2	W	E1 x E2	W
A1 x A3	W	C1 x C3	S	E1 x E3	O
A1 x A4	W	C1 x C4	W	E1 x E4	W
A2 x A3	S	C2 x C3	S	E2 x E3	O
A2 x A4	M	C2 x C4	M	E2 x E4	M
A3 x A4	O	C3 x C4	O	E3 x E4	M
B1 x B2	O	D1 x D2	S	F1 x F2	O
B1 x B3	W	D1 x D3	S	F1 x F3	W
B1 x B4	W	D1 x D4	M	F1 x F4	W
B2 x B3	M	D2 x D3	S	F2 x F3	S
B2 x B4	S	D2 x D4	W	F2 x F4	W
B3 x B4	O	D3 x D4	O	F3 x F4	O

* O=no chlorosis; W, weak; M, moderate; S, severe.

anum, but most of them were like the F_1 type, i.e., long, narrow, and pointed.

The Characters of the F_2 Generation

Sporidia were isolated from six F_1 chlamydospores from Cross 300 (cA2 x rB2) which had sori three cm. long. Monosporidial lines from individual chlamydospores were paired in all possible combinations and injected hydodermically into sorghum seedlings. The resulting sori were of different shapes and sizes. For example, 300D1 x 300D2 produced smutted heads resembling those of *Sphacelotheca cruenta;* 300A2 x 300A4 and 300A1 x 300A3 produced sori resembling those of long smut of sorghum caused by *Tolyposporium filiferum.* Crosses 300C1 x 300C2 and 300D2 x 300D3 produced sori resembling those of *Sorosporium reilianum.* Some of the monosporidial combinations (dicaryophytes) produced sori of the long type like those observed in the F_1, i.e., 300D1 x 300D2 produced sori 3 to 4 cm. long while 300E1 x 300E4 produced sori 6 to 7 cm. long (Plate 4). This indicates that different combinations of f_1 lines produce sori differing in size and shape and that grandparental types of sori disappear although the chlamydospores are echinulate in every F_2 type of sorus. The F_2 chlamydospores in various types of sori are, on an average, the same size as those of the F_1, and the manner of their germination also is like that of F_1 spores, i.e., by means of long promycelia with large sporidia or hyphal branches, or both.

Table 16. The Effect of Temperature on the Germination of F_2 Chlamydospores Resulting from Cross 400 (300D1 x 300D2), *(Sphacelotheca cruenta* x *Sorosporium reilianum)*

Temperatures in degrees C.	Percentage germination	Temperatures in degrees C.	Percentage germination
5	0	27½	60.4
10	9.2	30	64.3
15	16.7	35	32.4
20	30.3	40	12.5
25	42.6	45	4.7

Chlamydospores taken from an F_2 sorus of Cross 400 (300D1 x 300D2) were germinated in 2 per cent sucrose solution at various temperatures (Table 16). In temperature relations also the F_2 generation resembles the F_1 generation.

Sporidia was isolated from four chlamydospores of this F_2 generation (Cross 400) and studied for their cultural characters (Table 17). A cultural study of these f_2 lines shows that many of them are sporidial and resemble those of the p_1 lines *(Sphacelotheca cruenta* and *Sorosporium reilianum)* in color and consistency as well as margin and edge although few of these f_2 lines

Table 17. Cultural Characters of f_2 Lines from Promycelia of Four F_2 Hybrid Chlamydospores Resulting from Cross 400 (300D1 x 300D2), *(Sphacelotheca cruenta* x *Sorosporium reilianum)*

f_2 Line	Diameter in mm.	Color	Growth type*	Luster	Topography	Margin and edge	Number of sectors
A1	34	Brown with greenish center	S	Shiny	Flat	Dentate	0
2	32	Brown with greenish center	S	Shiny	Flat	Dentate	0
3	35	Brownish	S	Dull	Flat	Wavy	0
4	32	Brownish	S	Dull	Flat	Wavy	0
B1	35	Brown with greenish center	S	Shiny	Flat	Dentate	0
2	35	Brownish gray	S	Dull	Convex	Entire	2
3	40	Cinnamon	S	Dull	Flat	Undulating	0
4	40	Cinnamon	S	Dull	Flat	Undulating	0
C1	40	White surrounded by brown band	M	Dull	Convex	Entire	3
2	40	White surrounded by brown band	M	Dull	Convex	Entire	2
3	40	White surrounded by brown band	M	Dull	Convex	Entire	2
4	40	White surrounded by brown band	M	Dull	Convex	Entire	4
D1	35	Brownish gray	S	Shiny	Flat	Dentate	0
2	28	Brownish gray	S	Shiny	Convex	Dentate	1
3	40	Cinnamon	S	Shiny	Flat	Dentate	0
4	28	Brownish gray	S	Shiny	Convex	Dentate	1

* S=sporidial; M=mycelial.

Table 18. Size of Colonies and Numbers of Sectors in Four f_2 Lines from Cross 400 (300D1 x 300D2), (*Sphacelotheca cruenta* x *Sorosporium reilianum*), on Five Different Media

f_2 Line	Medium*				
	1	2	3	4	5
	Diameter of colonies in mm.				
A1	14	35	38	30	34
2	13	30	50	28	32
3	12	28	50	26	35
4	13	28	30	26	32
	Number of sectors				
A1	0	0	1	0	0
2	0	1	0	0	0
3	0	1	0	1	1
4	0	1	0	1	1

* 1. Agar 1.5 per cent.
2. Sucrose 3 per cent, agar 1.8 per cent, ammonium phosphate 0.012 per cent.
3. Peptone 1.5 per cent, dextrose 1.5 per cent, agar 1.5 per cent.
4. Malt extract 2 per cent, dextrose 1 per cent, agar 1.5 per cent.
5. Potato-dextrose agar 1.5 per cent.

are identical with f_1 lines. When four f_2 lines isolated from chlamydospore 400A were grown on five kinds of agar media in order to study the rate of growth and sectoring, they behaved more or less like f_1 lines with respect to growth. Line 400A1 sectored on Medium 3, line 400A2 on Medium 2, and lines 400A3 and 400A4 on media 2, 4, and 5 (Table 18).

Evidence of Heterosis

In 1928 Goldschmidt (16) hybridized some of the physiologic forms of *Ustilago violacea* and noticed that the promycelia of the hybrids were from 35 per cent to 75 per cent longer than those of the parents.

The writer (48), in his studies of the intergeneric hybrid between *Sphacelotheca sorghi* and *Sorosporium reilianum*, measured 50 promycelia of the parents and hybrid and pointed out that the promycelia of the hybrid are significantly larger than those of either parent, the mean lengths in *Sphacelotheca sorghi*, *Sorosporium reilianum*, and the hybrid being 24.65μ, 27.38μ, and 57.90μ, respectively. The sporidia of the hybrid also were statistically significantly longer than those of the parents.

In the present study the writer measured 100 chlamydospores and sporidia and 50 promycelia of *Sphacelotheca cruenta*, *Sorosporium reilianum*, and of the F_1 hybrid between them, and noticed that the chlamydospores were intermediate in size between both the parents but that the promycelia and sporidia were significantly larger than those of the parents. The mean lengths

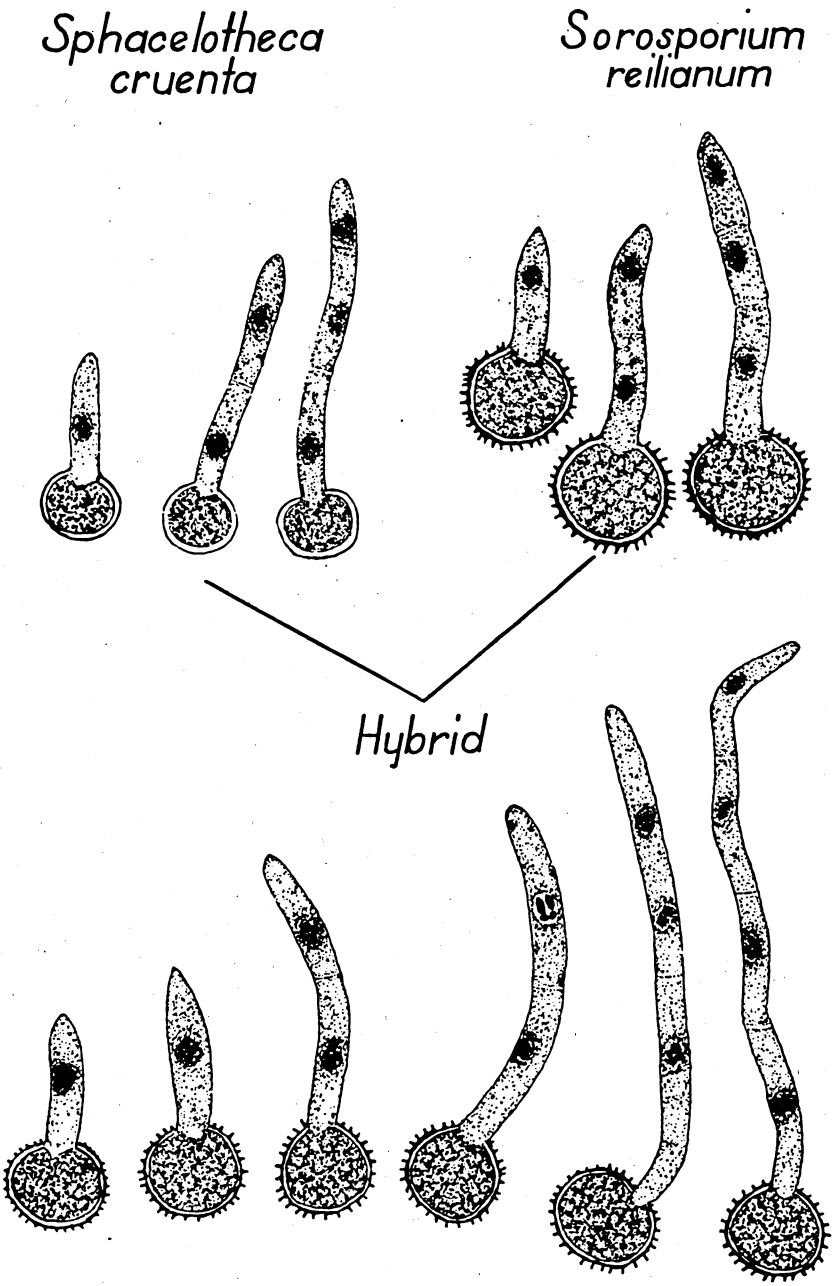

FIG. 4. GERMINATING CHLAMYDOSPORES OF *Sphacelotheca cruenta*, *Sorosporium reilianum*, AND THE F_1 HYBRID SPORES, SHOWING NUCLEI. THE DARK BODIES IN THE NUCLEI MAY BE CHROMOSOMES

of promycelia of the *Sphacelotheca cruenta, Sorosporium reilianum,* and the hybrid were 25.21μ, 27.38μ, and 79.0μ, respectively, while the mean lengths of sporidia were 11.26μ, 5.64μ, and 12.7μ, respectively (Table 8). This shows that there is pronounced heterosis in this intergeneric hybrid.

There is a further evidence of heterosis in the long sori obtained in the F_1 and F_2 generations.

More of the hybrid chlamydospores germinate at a higher temperature than either parent, which may possibly be due to heterosis.

The f_1 lines grew very much better on Medium 2 (1.8 per cent agar, 3 per cent sucrose, and 0.012 per cent ammonium phosphate). Evidently, therefore, the f_1 lines are more vigorous, at least on some substrata.

Cytologic Studies

Hanna (18) showed that the chlamydospores, promycelial cells, and the sporidia of *Sorosporium reilianum* are uninucleate. According to Rodenhiser (37), the nuclear behavior in the germinating chlamydospores of *Sphacelotheca sorghi* and *S. cruenta* is essentially the same and similar to that in *Ustilago zeae.* The mature chlamydospore contains a single diploid nucleus which divides into two daughter nuclei in the promycelium, and these daughter nuclei divide again, thus forming four nuclei. This general sequence was observed by the writer also in *Sphacelotheca cruenta, Sorosporium reilianum,* and the hybrid.

Germinating chlamydospores of *Sphacelotheca cruenta, Sorosporium reilianum,* and the hybrid were stained in Haidenhain's iron-alum haematoxylin (7) in order to study the nuclear condition in the promycelia. In earlier stages in the promycelia of *Sphacelotheca cruenta, Sorosporium reilianum,* and the hybrid, four dark staining bodies were observed in the nuclei. In late stages, when the promycelia had divided into two segments, four dark staining bodies also were observed; and in a still later stage, when the promycelia had more than two segments, two to four dark staining bodies were seen, suggesting a diploid and haploid condition (Fig. 4). These dark staining bodies appeared to be chromosomes, but further work must be done before this can be stated with certainty.

DISCUSSION AND CONCLUSIONS

BECAUSE there are four species of smut fungi that attack sorghum, it seems desirable to ascertain to what extent new biotypes might arise in nature as a result of intraspecific and interspecific or intergeneric hybridization.

A study of *Sphacelotheca sorghi* revealed that this species comprises a great many haploid biotypes that differ in sex and in cultural characters. The 74 haploid derivatives from 28 chlamydospores grouped themselves into about 16 types on the basis of cultural characters. There apparently are at least 60 sex groups among the 74 haploid lines. This was determined by the use of the Bauch test which had been shown by Tyler to be entirely reliable for sex determination in *S. sorghi.* The writer confirmed Tyler's conclusion and confirmed the results of the Bauch test with the results of other available tests for sex determination, viz., fusion of sporidia, the production of chlorosis in inoculated sorghum plants, and the final development of chlamydospores by sexually compatible lines. The conclusion seems justified, therefore, that there are a very large number of sexual groups in *S. sorghi.* If haploid lines are sexually compatible, they hybridize freely and produce new morphologic and physiologic types.

Peridial color and, consequently, color of smutted heads appear to be due to genetic factors. When monosporidial lines from chlamydospores obtained from brown heads were crossed, the smutted heads were brown. The brown x brown crosses were continued to the sixth generation and only brown heads appeared.

When haploid lines from chlamydospores of the gray type were crossed, however, two types of heads resulted, brownish gray and gray. When a brown x gray cross was made, the F_1 head type was brownish gray, and crosses between haploid lines from this head type produced brown, brownish-gray, and gray head types. Obviously this should not have been the case when only two monosporidial lines were crossed, because all dicaryons should have been exactly the same, barring undetected mutation or unusual nuclear distribution. There are three possible explanations:

1. The variety of sorghum used did not comprise a pure line, and consequently certain individuals within the variety may have had a tendency to produce certain colors.

2. Mutation of the pathogen may have occurred in the sorghum plants or prior to their inoculation.

3. The factors for brown and gray in brown x gray dicaryophytes may have been so delicately balanced as to account for considerable variation in the color.

The last explanation seems more probable in view of the fact that no color other than brown appeared in the brown x brown crosses.

There was clear-cut evidence for differences in pathogenicity of haploid lines. Various dicaryophytes produced different degrees of chlorosis, the precise significance of which is not yet known. It seems probable that a preliminary indication of the degree of pathogenicity can be obtained by the degree of chlorosis produced on sorghum plants. Conclusive evidence, however, was obtained that dicaryophytes with different pathogenic capabilities may arise as a result of the union of haploid lines. One dicaryophyte (Cross 124) would have to be designated a new physiologic race, differing in pathogenicity from the five hitherto described by various investigators.

Because of these facts, it is well to be prepared for changes in varietal resistance. The writer, therefore, made studies of control measures other than the development of resistant varieties. It was found in the case of *Sphacelotheca sorghi* that early planting, when soil temperature is likely to be low, tends to decrease the amount of smut. It also was found as a result of testing a large number of fungicides that the covered kernel smut *(S. sorghi)* can be completely controlled by treating the seed with New Improved Ceresan. Ceresan, Koppers "Flotation" sulphur, wettable sulphur, powdered flowers of sulphur, gas sulphur, and Kolo dust were also effective in controlling the smut. These fungicides did not cause seed injury during the two years of the test.

It had been shown by other investigators that interspecific and intergeneric hybridization was possible in the sorghum smuts. The writer therefore paired five haploid lines of *Sphacelotheca cruenta* with seven haploid lines of *Sorosporium reilianum* in 18 combinations, and all of them produced infection, indicating that hybridization between these two genera takes place readily. The hybrids were fertile, no indication of sterility appearing in subsequent studies. Some of the F_1 sori resembled one parent, and others resembled the other. There also were a number of intermediate types, but, most remarkable, there were a number of sori that resembled those caused by *Tolyposporium filiferum*. Some of them were 7 cm. in length, indicating that the dicaryophytes

causing them stimulated pronounced hypertrophy in the affected tissues. The F_1 chlamydospores were intermediate between those of the parents, but they germinated better than those of either parent at high temperatures, in this respect resembling those of *T. filiferum*. There was marked evidence of heterosis in promycelia, sporidia, and hyphal branches growing from the promycelia. Whereas the promycelia of the parents are usually approximately 25μ long, those of the hybrid chlamydospores sometimes exceeded 75μ, and the sporidia were correspondingly large. Furthermore, some of the haploid lines derived from F_1 chlamydospores tolerated ammonium phosphate in the culture medium far better than any of those of the parents. Studies of the F_2 generation substantiate, in general, the results recorded for the F_1 generation.

The results show clearly that there are many freely-interbreeding haploid biotypes within the species of sorghum smuts studied and that hybridization between biotypes of different genera can take place readily. Different morphologic types of smut sori may result, dicaryophytes differing in pathogenicity may result; and, in consequence, hybridization may have far-reaching implications in the taxonomy and pathogenicity of the sorghum smuts.

SUMMARY

1. From a single sorus of a Texas collection of *Sphacelotheca sorghi* three chlamydospores were germinated, single sporidia were isolated, and the resulting haploids were crossed. One cross resulted in the production of brown heads in inoculated sorghum plants and the other produced gray heads. Four crosses were made between f_1 lines, two within the brown type, and two between brown and gray.

2. From the "intrabrown" and brown x gray crosses 28 chlamydospores were germinated, and segregation of factors for cultural characters and sex was studied. Ratios for segregation of factors for cultural characters were 4:0, 3:1, 2:2, 1:2:1, and 1:1:1:1; while those observed for sex were 2:2, 1:2:1, and 1:1:1:1. Segregation of factors for cultural characters and for sex apparently was independent.

3. The Bauch test proved to be a reliable method of determining sex in *Sphacelotheca sorghi*, as is evident from the results of this test compared with those obtained by inoculating sorghum.

4. When 24 monosporidial lines, isolated from six chlamydospores, were paired in all possible combinations, 24 sexual groups were found. Furthermore, when 74 monosporidial lines isolated from 28 chlamydospores were paired in all possible combinations, as many as 60 sexual groups were observed. There appear, therefore, to be multiple factors for sex.

5. Different combinations (dicaryophytes) of sexually compatible monosporidial lines produced different degrees of chlorosis on the leaves of sorghum plants, indicating that different dicaryophytes differ in pathogenicity.

6. *Sphacelotheca sorghi* with brown peridial wall bred true, while the type with gray peridial wall produced gray and brownish-gray types. It seems likely that there may be multiple factors for the inheritance of gray color.

7. The percentage of infection of sorghum with *S. sorghi* was fairly high when seed germinated at an average soil temperature between 50° F. and 70° F., while at a lower temperature there was a marked decrease in infection. Planting in the fourth week of April or first week of May will possibly reduce infection considerably.

8. The reaction of 48 varieties of sorghum to a collection of smut (Collection 124), which is the F_2 progeny of a cross between two biotypes, is discussed. Collection 124 differed in pathogenicity from the five physiologic races hitherto described.

9. It is evident that Collection 124 is a new physiologic race of *S. sorghi.* It was designated as Race 6. As it attacks hegari moderately and Reed kafir severely, it could be designated the hegari-kafir race. A new key for the identification of all the six races is given.

10. Among the various seed disinfectants tried for controlling *S. sorghi,* New Improved Ceresan prevented the smut entirely. Ceresan, Koppers "Flotation" sulphur, wettable sulphur, powdered flowers of sulphur, gas sulphur, and Kolo dust also were effective.

11. There was no reaction in the Bauch test when haploid lines from *S. sorghi* or *S. cruenta* were mixed with haploid lines of *Sorosporium reilianum.* Similarly, no sporidial fusions were observed when monosporidial lines from *Sphacelotheca sorghi* or *S. cruenta* were mixed with monosporidial lines of *Sorosporium reilianum.*

12. There is evidence that sexual compatibility of monosporidial lines from *Sphacelotheca cruenta* and *Sorosporium reilianum* can be detected by inoculating sorghum plants with them. Sex-

ually compatible lines produce chlorosis 8 to 12 days after inoculation, and chlorotic plants later develop sori containing chlamydospores.

13. *Sphacelotheca cruenta* and *Sorosporium reilianum* are interfertile. Sexually compatible monosporidial lines of these two genera, when paired, can cause infection.

14. Different monosporidial combinations of the two genera produced sori differing in shape and size. Some of the sori resemble those of *Sphacelotheca cruenta*, some resemble those of *Sorosporium reilianum*, while some resemble those of *Tolyposporium filiferum*; and some sori are very narrow and long, measuring up to 7 cm.

15. The F_1 chlamydospores of the hybrid are echinulate and intermediate in size, the promycelia and sporidia being significantly larger than those of the parents.

16. The hybrid chlamydospores have a higher optimum temperature for germination than the parents.

17. The ratios of segregation of factors for cultural characters in the f_1 lines of the hybrid were 4:0, 3:1, 2:2, 1:2:1, and 1:1:1:1; while those for segregation of factors for sex were 2:2, 1:2:1, and 1:1:1:1. Monosporidial lines from two promycelia fell into seven sex groups when paired in all possible combinations.

18. Variants appeared in monosporidial cultures of the hybrid which were culturally distinct from the parent colonies.

19. Different paired combinations of the f_1 lines of the hybrid produced different degrees of chlorosis (severe, moderate, and weak) on sorghum leaves when inoculated hypodermically into sorghum seedlings. Monosporidial lines alone caused no infection. Chlorotic plants later produced sori. This shows that the hybrid is heterothallic and can cause normal infection in the host, and indicates that different combinations of sporidial lines have different degrees of pathogenicity.

20. The F_2 sori produced as a result of different combinations of f_1 lines differ in size and shape. Some of the F_2 sori resemble the grandparental types while others are either like *Tolyposporium filiferum* or like some of the F_1 types, with very narrow and long sori. The F_2 chlamydospores are echinulate and germinate by long promycelia like those of the F_1, bearing sporidia or hyphal branches or both.

21. The F_2 chlamydospores have more or less the same temperature relations for germination as the F_1 chlamydospores.

22. Some of the f_2 lines cultured on potato-dextrose agar resemble the colonies of some haploid lines of *Sphacelotheca cruenta*

and of *Sorosporium reilianum,* while others resemble the colonies of f_1 lines.

23. Heterosis, like that in higher plants, is evident in this intergeneric hybrid.

24. From cytologic studies of nuclei in the promycelia of *Sphacelotheca cruenta, Sorosporium reilianum,* and the hybrid there is evidence that there are four chromosomes in diploid nuclei and two in haploid nuclei.

LITERATURE CITED

1. ALLISON, C. C. Hybridization between *Ustilago hordei* and *U. medians.* (Abst.) Phytopath. **25:**5. 1935.
2. BAUCH, R. Über *Ustilago longissima* und ihre Varietät *macrospora.* Ztschr. f. Bot. **15:**241-279. 1923.
3. ———— Über multipolare Sexualität bei *Ustilago longissima.* Arch. f. Protistenk. **70:**417-466. 1930.
4. ———— Die Sexualität von *Ustilago scorzonerae* und *Ustilago zeae.* Phytopath. Ztschr. **5:**315-321. 1932.
5. BRITON-JONES, H. R. The smuts of millet. Egypt Min. Agr. Tech. and Sci. Serv. Bul. **18:** (Bot. section). 1922.
6. BUTLER, E. J. Fungi and disease in plants. 547 pp. Thacker, Spink & Co., Calcutta and Simla. 1918.
7. CHAMBERLAIN, C. J. Methods in plant histology. Ed. 5, 416 pp. University of Chicago Press, Chicago. 1932.
8. CHRISTENSEN, J. J. Mutation and hybridization in *Ustilago zeae.* Minn. Agr. Expt. Sta. Tech. Bul. 65. Pt. **2:** Hybridization. 1929.
9. ———— Studies on the genetics of *Ustilago zeae.* Phytopath. Ztschr. **4:**129-188. 1931.
10. DICKINSON, S. A method of isolating and handling individual spores and bacteria. Roy. Soc. Med. London, Proc. **19:**1-4. 1926.
11. ———— Experiments on the physiology and genetics of the smut fungi. Hyphal fusion. Roy. Soc. London, Proc. Ser. B, **101:**126-136. 1927.
12. ———— Experiments on the physiology and genetics of the smut fungi. Cultural characters. I. Their permanence and segregation. Roy. Soc. London, Proc. Ser. B, **103:**547-555. 1928.
13. EDDINS, A. H. Pathogenicity and cultural behavior of *Ustilago zeae* (Bekm.) Ung. from different localities. Phytopath. **19:**885-916. 1929.
14. FLOR, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis.* Jour. Agr. Res. **44:**49-58. 1932.
15. FREEMAN, E. M., and UMBERGER, H. J. C. The smuts of sorghum. U.S. Dept. Agr. Bur. Pl. Ind. Circ. **8:** 1908.
16. GOLDSCHMIDT, VICTOR. Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violacea* Pers.) Ein Beitrag zur Frage der parasitären Spezialisierung. Ztschr. f. Bot. **21:**1-90. 1928.

17. HANNA, W. F. A simple apparatus for isolating single spores. Phytopath. **18**:1017-1021. 1928.

18. ———— Studies in the physiology and cytology of *Ustilago zeae* and *Sorosporium reilianum*. Phytopath. **19**:415-442. 1929.

19. ———— and POPP, W. Relationship of the oat smuts. Nature **126**:843-844. 1930.

20. HOLTON, C. S. Studies in the genetics and the cytology of *Ustilago avenae* and *Ustilago levis*. Minn. Agr. Expt. Sta. Tech. Bul. 87. 1932.

21. ISENBECK, K. Untersuchungen über die Physiologie von *Sphacelotheca sorghi* den gedeckten Körnerbrand von Sorghum. Phytopath. Ztschr. **8**:165-182. 1935.

22. JOHNSTON, C. O., and MELCHERS, L. E. The control of sorghum kernel smut and the effect of seed treatments on vitality of sorghum seed. Kans. Agr. Expt. Sta. Tech. Bul. 22. 1928.

23. KAMAT, M. N. Observations on *Tolyposporium filiferum*, cause of "long smut" of sorghum. Phytopath. **23**:985-992. 1933.

24. KNIEP, HANS. Untersuchungen über den Antherenbrand (*Ustilago violacea* Pers.) Ein Beitrag zum Sexualitätoproblem. Ztschr. f. Bot. **11**:257-284. 1919.

25. ———— Über Artkreuzungen bei Brandpilzen. Ztschr. Pilzk. **5**:217-247. 1926.

26. KULKARNI, G. S. Smuts of Jowar (sorghum) in the Bombay Presidency. Pusa Agr. Res. Inst. Bul. 78. 1918.

27. ———— Conditions influencing the distribution of grain smut *(Sphacelotheca sorghi)* of Jowar (sorghum) in India. Agr. Jour. India **17**:159-162. 1922.

28. MCALPINE, D. The smuts of Australia. Their structure, life history, treatment and classification. 288 pp. J. Kemp, Government Printer, Melbourne. 1910.

29. MELCHERS, L. E., FICKE, C. H., and JOHNSTON, C. O. Physiologic specialization in *Sphacelotheca sorghi*. (Abst.) Phytopath. **20**:142-143. 1930.

30. ———— FICKE, C. H., and JOHNSTON, C. O. A study of the physiologic forms of kernel smut *(Sphacelotheca sorghi)* of sorghum. Jour. Agr. Res. **44**:1-11. 1932.

31. PORTER, R. H. A preliminary report of surveys for plant diseases in East China. The Plant Disease Reporter Suppl. **46**:153-166. 1926.

32. POTTER, A. A. The loose kernel smut of sorghum. Phytopath. **5**:149-154. 1915.

33. ———— and MELCHERS, L. E. Resistance of sorghum types to covered kernel smut (Abst.). Phytopath. **8**:71. 1918.

34. REED, G. M., and FARIS, J. A. Influence of environmental factors on the infection of sorghums and oats by smuts. I. Experiments with covered and loose kernel smuts of sorghum. Amer. Jour. Bot. **11**:518-534. 1924.

35. ———— and MELCHERS, L. E. Sorghum smuts and varietal resistance in sorghums. U. S. Dept. Agr. Bul. 1284. 1925.

36. RODENHISER, H. A. Heterothallism and hybridization in *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. **45**:287-296. 1932.

37. ———— Studies on the possible origin of physiologic forms of *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. **49**:1069-1086. 1934.

38. SHUMWAY, C. P. Studies in the cytology and genetics of *Sorosporium reilianum* (Kühn) McAlpine. Unpublished M.S. thesis, University of Minnesota. Filed in Library, Univ. of Minn. 1931.

39. SLEUMER, H. O. Über Sexualität und Zytologie von *Ustilago zeae* (Bekm.) Unger. Ztschr. f. Bot. **25**:209-263. 1932.

40. SNEDECOR, G. W. Calculation and interpretation of analysis of variance. 96 pp. Collegiate Press, Inc., Ames, Iowa. 1934.

41. STAKMAN, E. C., and CHRISTENSEN, J. J. Heterothallism in *Ustilago zeae*. Phytopath. **17**:827-834. 1927.

42. ———— CHRISTENSEN, J. J., EIDE, C. J., and PETURSON, BJORN. Mutation and hybridization in *Ustilago zeae*. Minn. Agr. Expt. Sta. Tech. Bul. 65. 1929.

43. ———— TYLER, L. J., and HAFSTAD, G. E. The constancy of cultural characters and pathogenicity in variant lines of *Ustilago zeae*. Torrey Bot. Club Bul. **60**:565-572. 1933.

44. TISDALE, W. H., MELCHERS, L. E., and CLEMMER, H. J. Strains of kernel smuts of sorghum, *Sphacelotheca sorghi* and *S. cruenta*. Jour. Agr. Res. **34**:825-838. 1927.

45. TYLER, LEON J. Methods for determining sex differences in *Sphacelotheca sorghi* (Link) Clinton. Phytopath. **24**:691-693. 1934.

46. ———— Variation in *Sphacelotheca sorghi* (Link) Clinton. Minn. Agr. Expt. Sta. Tech. Bul. 133. 1938.

47. ———— and SHUMWAY, C. P. Hybridization between *Sphacelotheca sorghi* and *Sorosporium reilianum*. Phytopath. **25**:375-376. 1935.

48. VAHEEDUDDIN, SYED. Hybridization and segregation in crosses between *Sphacelotheca sorghi* and *Sorosporium reilianum*. Unpublished M.S. thesis, University of Minnesota. Filed in the Library, Univ. of Minn. 1935.

49. ———— Hybridization between *Sphacelotheca cruenta* and *Sorosporium reilianum* (Abst.). Phytopath. **26**:111. 1936.

50. ———— The production of a new physiologic race of *Sphacelotheca sorghi*. Phytopath. **28**:656-659. 1938.

51. ZILLIG, HERMAN. Über spezialisierte Formen beim Antherenbrand, *Ustilago violacea* (Pers.) Fuck. Centralbl. Bakt. II. **53**:33-74. 1921.

PLATES

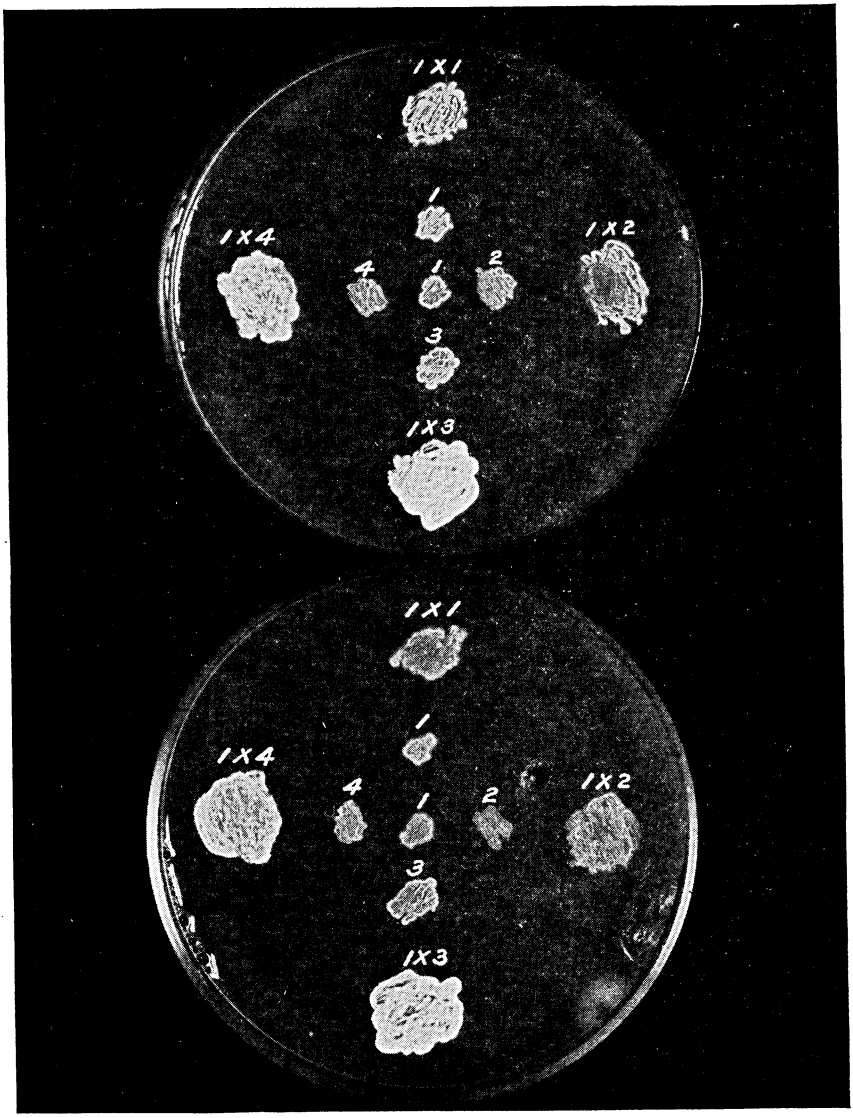

PLATE 1. DUPLICATE PLATES SHOWING THE RESULTS OF THE BAUCH TEST ON FOUR MONOSPORIDIAL LINES FROM A SINGLE CHLAMYDOSPORE, 23A, OF *Sphacelotheca sorghi*

Sporidium 1 is the tester. Material from the center colony was smeared on the colonies of lines 1, 2, 3, and 4 in the outer circle, while the colonies of the same lines in the inner circle were left as checks. It is evident that sporidia 1 and 2 are of sex different from sporidia 3 and 4, as indicated by cottony growth of "Suchfäden." Combination 1 x 3 shows a dense cottony growth of "Suchfäden," while combination 1 x 4 shows a weak growth of "Suchfäden." Confirmatory results were obtained by sporidial fusions and by the production of chlorosis on leaves injected hypodermically.

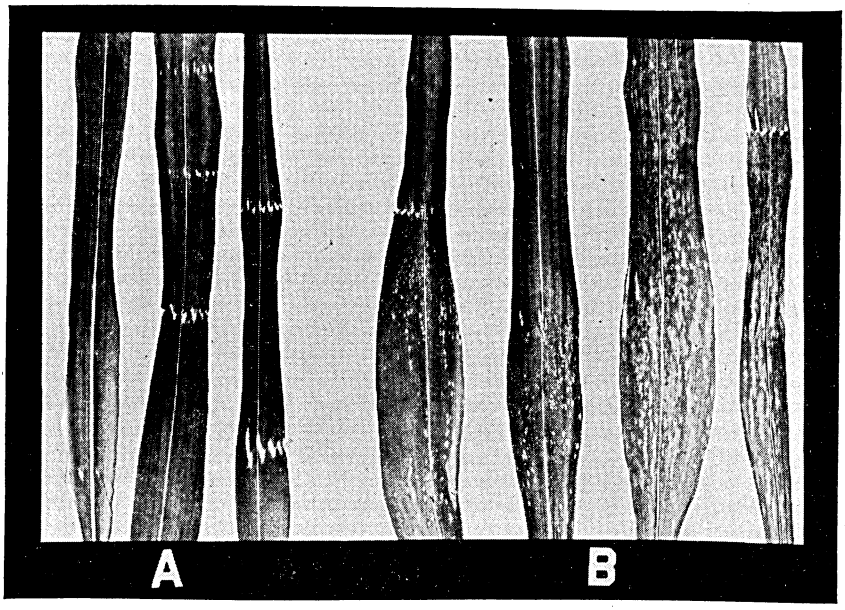

PLATE 2. LEAVES FROM SORGHUM PLANTS INOCULATED WITH SEXUALLY COMPATIBLE AND INCOMPATIBLE COMBINATIONS OF MONOSPORIDIAL LINES OF *Sphacelotheca sorghi*

A shows needle punctures on leaves but no chlorosis, i.e., sexually incompatible combination of monosporidial lines; B shows needle punctures as well as chlorosis caused by sexually compatible combination of monosporidial lines.

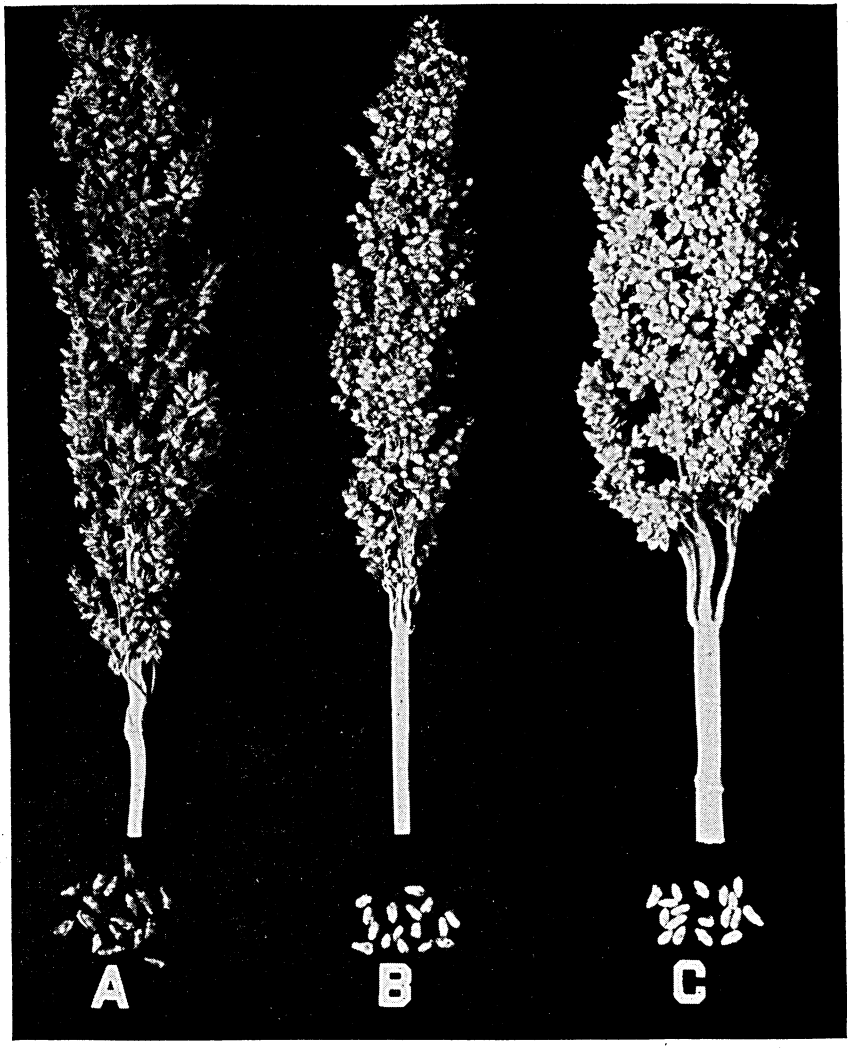

PLATE 3. HEADS OF SMUTTED MINNESOTA AMBER SORGHUM, SHOWING BROWN, BROWNISH-GRAY, AND GRAY PERIDIA

A. Brown, B. Brownish gray, and C. Gray.

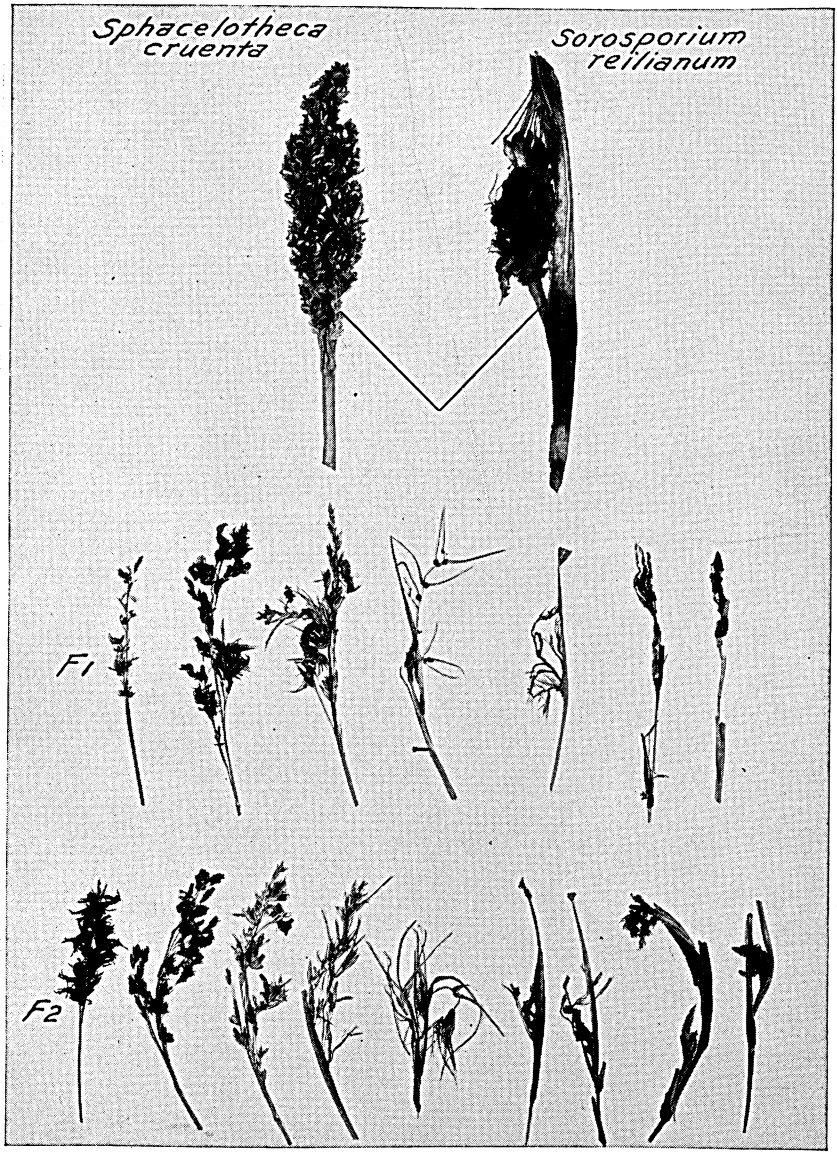

PLATE 4. SMUTTED HEADS OF SORGHUM: PARENTAL TYPES—*Sphacelotheca cruenta* AND *Sorosporium reilianum*, AND F_1 AND F_2 SORI

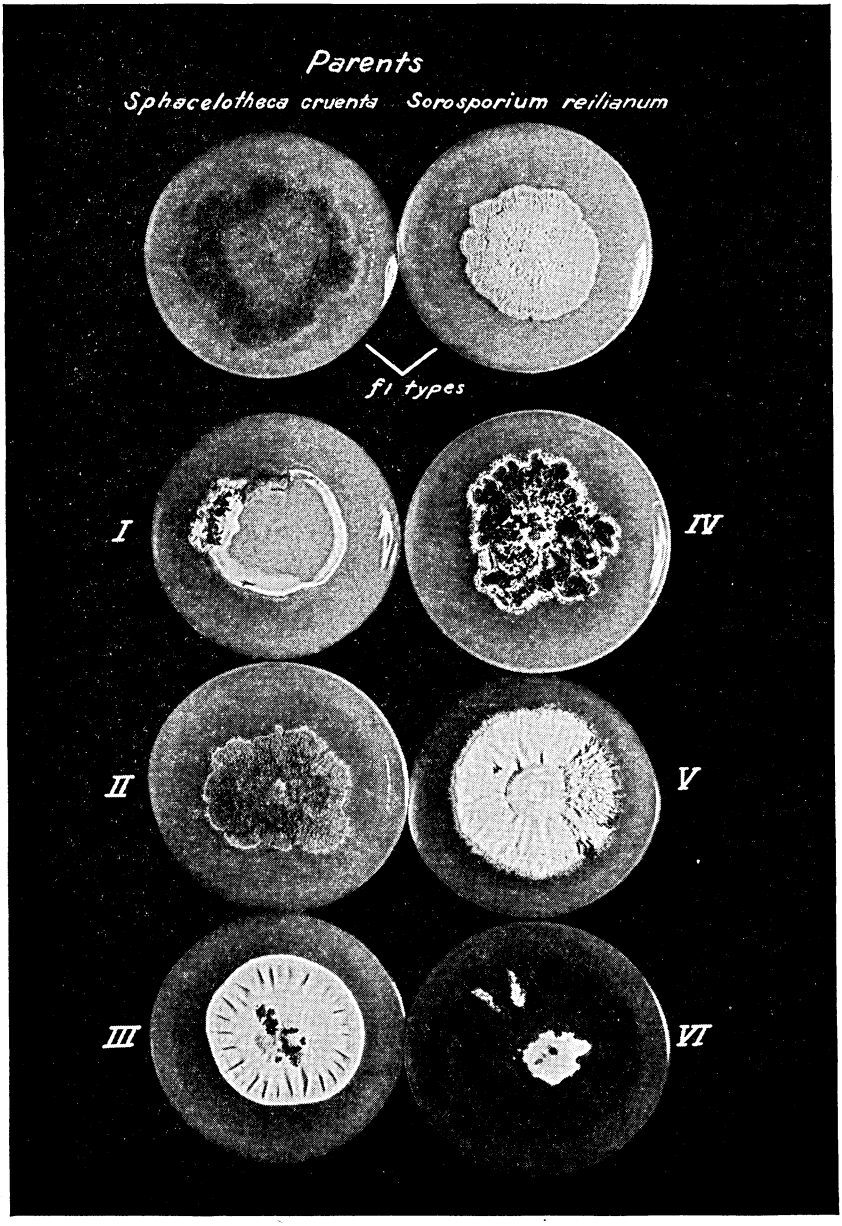

PLATE 5. PARENTS (ABOVE) AND SIX f_1 LINES (I TO VI) RESULTING FROM CROSS 300 (*Sphacelotheca cruenta* [cA2] x *Sorosporium reilianum* [rB2])

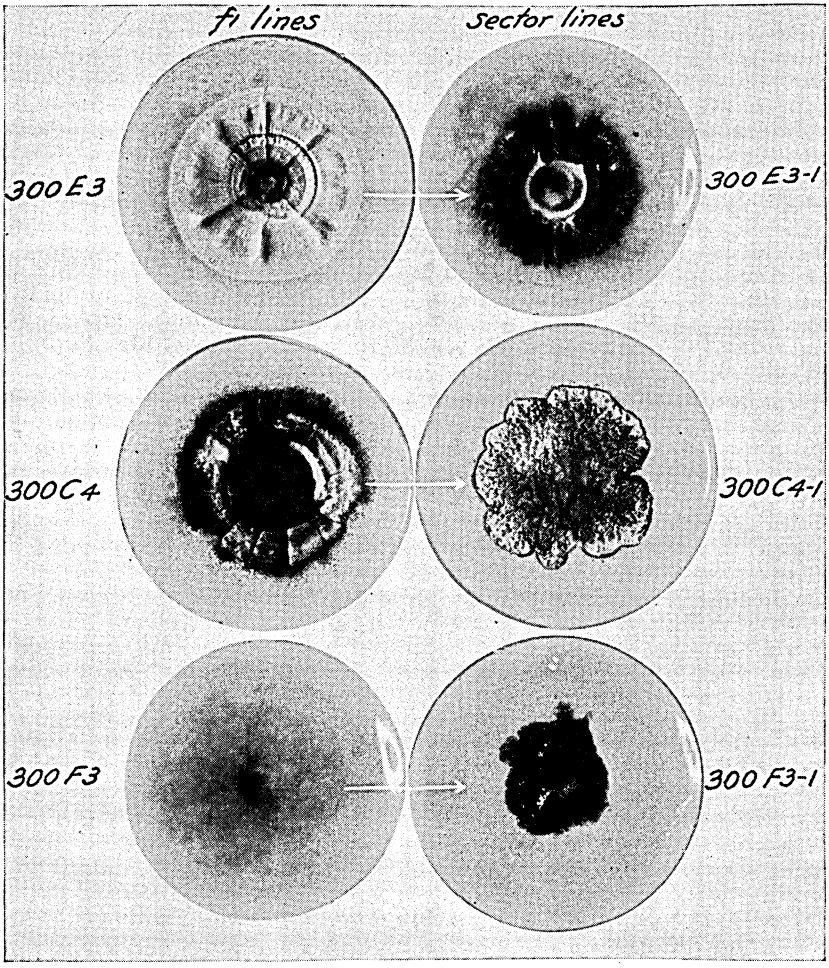

PLATE 6. LEFT, THREE f_1 LINES FROM CROSS 300 (*Sphacelotheca cruenta* [cA2] x *Sorosporium reilianum* [rB2]); RIGHT, A VARIANT FROM EACH LINE